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PULP AND PAPER ENVIRONMENTAL EFFECTS MONITORING (EEM) PROGRAM IN THE ATLANTIC REGION

CYCLE 2 SYNOPSIS REPORT

(April 1996 – March 2000)

Surveillance Report EPS-5-AR-02-02
Atlantic Region

Canada

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Pulp and Paper Environmental Effects Monitoring Program in the Atlantic Region

Cycle 2 Synopsis Report
(April 1996 – March 2000)

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Environmental Protection Branch
Environment Canada
Atlantic Region

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EXECUTIVE SUMMARY

In May 1992, the 1971 Pulp and Paper Effluent Regulations (PPER) under the Fisheries Act were amended. The amendments included stricter effluent discharge limits on total suspended solids (TSS), biochemical oxygen demand (BOD) and effluent toxicity, and a requirement for every regulated pulp and paper mill in Canada subjected to the PPER to conduct an environmental effects monitoring (EEM) program. The objective of the EEM was to determine if fish, fish habitat and the use of fisheries resources were being adequately protected by the requirements of the PPER. The first EEM Cycle was completed on April 01, 1996. Results from the second EEM Cycle were submitted on April 01, 2000.

The objective of Cycle 1 was to establish environmental conditions at sites, to evaluate variability in the EEM endpoints and to assess various methodologies/tools used in EEM. Using the information gleaned from Cycle 1, studies were designed in Cycle 2 to test the null hypothesis that effluents from the mills were not affecting the biota in the receiving environment. The Cycle 2 study components in the Atlantic Region included: a fish survey, an invertebrate community survey, supporting water and sediment quality measurements, and sublethal toxicity tests.

There were 19 mills operating in the Atlantic Region during Cycle 2. A total of 18 studies were conducted and reported (17 individual studies and 1 joint EEM study). The Cycle 2 data from the Atlantic Region indicate that there are statistical differences in various endpoints measured between exposed and reference sites for several mills. However, at many of these sites, due to confounding factors and our lack of understanding of the natural variability in the endpoints that were measured, the differences could not be attributed solely to mill effluents. Improved study designs and alternative studies are required in Cycle 3 to better assess the effect of pulp mill effluent discharges on the receiving environment.

A comparison of Cycle 1 and Cycle 2 sublethal toxicity data for all organisms tested indicated a great improvement in effluent quality in Cycle 2. The toxicity of effluents was greatly reduced in Cycle 2 in all organisms tested. Most notable was that the majority of effluents discharging into marine/estuarine receiving environment showed no sublethal effects in the 7-day fish test. Despite this reduction in sublethal toxicity, the effluent from most of the mills in the Atlantic Region still elicits toxic responses in invertebrates and algae.

This report summarizes the results of the 18 EEM studies, outlines areas where problems were encountered, and provides recommendations for changes in the EEM program.

RÉSUMÉ

En mai 1992, le *Règlement sur les effluents des fabriques de pâtes et papiers* (REFPP) de 1971, pris en application de la *Loi sur les pêches*, a été modifié. Les modifications fixaient des limites plus strictes pour le total des solides en suspension (TSS), la demande biologique en oxygène (DBO) et la toxicité des effluents, et exigeait de chaque fabrique qu'elle réalise un programme d'étude de suivi des effets sur l'environnement (ESEE). L'objectif de l'ESEE était de déterminer si le poisson, son habitat et l'utilisation des ressources halieutiques étaient correctement protégés par les exigences du REFPP. Le premier cycle d'ESEE a été complété le 1er avril 1996. Les résultats du second cycle d'ESEE ont été présentés le 1er avril 2000.

Le cycle 1 avait pour objet d'établir les conditions environnementales existant sur le site, de mesurer la variabilité des mesures terminales de l'ESEE et d'évaluer diverses méthodologies et divers outils employés dans l'ESEE. À l'aide de l'information recueillie pendant le cycle 1, on a conçu pour le cycle 2 des études de vérification de l'hypothèse nulle suivante : les effluents des fabriques n'ont aucun impact sur le biote du milieu récepteur. Dans la région atlantique, le cycle 2 se composait des éléments suivants : une étude des poissons, une étude de la communauté d'invertébrés benthiques, une évaluation des mesures complémentaires de la qualité de l'eau et des sédiments et de la toxicité sublétale.

On comptait 19 fabriques en opération dans la région atlantique pendant le cycle 2. Au total, 18 études d'ESEE ont été menées et ont fait l'objet d'un rapport (17 études individuelles et une étude conjointe). Les données du cycle 2 de la région atlantique montrent qu'il existe des différences dans les diverses mesures terminales entre les sites exposés et les sites de référence pour plusieurs fabriques. Toutefois, à bon nombre de ces sites, les différences n'ont pas pu être attribuées uniquement aux effluents des fabriques à cause des facteurs confondants et d'un manque de connaissances au sujet de la variabilité naturelle de ces mesures terminales. L'amélioration de la conception d'étude et des études alternatives permettra de mieux évaluer l'effet des rejets actuels sur le milieu récepteur.

La comparaison des données du cycle 1 et du cycle 2 sur la toxicité sublétale pour tous les organismes testés a fait ressortir une nette amélioration de la qualité de l'effluent au cycle 2. La toxicité de l'effluent était fortement réduite au cycle 2 chez tous les organismes testés, la majorité des effluents rejetés dans le milieu marin ou estuarien ne décelant aucun effet sublétal sur le poisson (essai de 7 jours). Malgré cette réduction de la toxicité sublétale, l'effluent de la plupart des fabriques de la région atlantique suscite encore des réactions toxiques chez les invertébrés et les algues.

Le rapport résume les résultats des 18 études d'ESEE, signale les secteurs où des problèmes ont été notés, et fait des recommandations de modifications à apporter au Programme de l'ESEE.

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1.0 INTRODUCTION

In May 1992, the 1971 Pulp and Paper Effluent Regulations (PPER) under the *Fisheries Act* were amended. The amendments included stricter limits on biochemical oxygen demand, total suspended solids and acute toxicity of effluent to fish. In addition to the changes in effluent limits, the amendments also required the mills to conduct an environmental effects monitoring (EEM) program. Under the EEM program, the mills were required to determine the effects of their effluent on fish, fish habitat and the use of fisheries resources. All mills were required to be in compliance with the amended regulations by December 31, 1995.

The EEM program is a sequential series of monitoring and interpretation cycles, where the requirements of each cycle are dependent on the findings of the previous cycle (Humphrey and Ali, 1997). The EEM studies were to be conducted on a three year cycle with the Cycle 1 report due on April 1, 1996; however, after the first cycle, a one year review period was established to assess the Cycle 1 results by a series of expert working groups. As a result, an amendment to PPER was made to extend the cycle to a 4-year cycle in 1997. The Cycle 2 reports were due on April 01, 2000 and the Cycle 3 reports are due on April 01, 2004. All pulp and paper mills in the Atlantic Region completed their Cycle 2 studies and submitted their reports to Environment Canada by April 01, 2000 as required. This report covers the period from April 1996 to March 2000.

The objectives of EEM are to determine if fish, the fish habitat and the utilization of the fisheries resources are being adequately protected by the requirements of the PPER. The requirements for the EEM studies are set out in EEM Requirements Document, Annex 1 and the Pulp and Paper EEM Technical Guidance Document (TGD) (Environment Canada, 1998).

In Cycle 2, all the mills in the Atlantic Region conducted a benthic invertebrate community study, sublethal toxicity tests, and took supporting water and sediment quality measurements (Table 1). Six mills attempted a fish study, with only five being able to catch sufficient number of at least one species of fish; five mills conducted research projects (alternative methods to the traditional fish survey); and the remaining eight mills did not conduct a fish or fish alternative studies. The dioxin and furan analyses, and tainting studies were not required at any of the mills in the Atlantic Region.

This report provides a summary of the results obtained from the Cycle 2 EEM studies conducted by the mills in the Atlantic Region and suggests recommendations for future EEM cycles.

1.1 Administration of EEM Program in the Atlantic Region

The EEM Program is a national program administered by Environment Canada as part of the PPER under the *Fisheries Act*. EEM program is administered regionally by the Regional Authorization Officer (RAO) on behalf of the Minister. The RAO for the Atlantic Region for Cycle 2 was the Regional Director of Environmental Protection Branch, Environment Canada.

Mills are required to submit EEM study designs to the RAO 180 days before any field studies are initiated. The study designs are reviewed by the Technical Advisory Panel

(TAP) and approved by the RAO before field studies are conducted. After field studies are conducted, the mills are required to submit EEM interpretive reports to the RAO. The reports are approved and accepted by the RAO based on recommendations made by the TAP in the Atlantic Region.

The role of the TAP is to review the proposed study designs and methodologies, and to provide suggestions/recommendations to improve the studies in order to address the objectives of EEM. The TAP also reviews the interpretive reports, and provides suggestions and recommendations on the interpretation of the data and direction for future studies. In the Atlantic Region, there are 3 TAPs: New Brunswick TAP, Nova Scotia TAP and Newfoundland TAP. Each TAP consists of representatives from the Oceans Branch of Department of Fisheries and Oceans, Provincial Department of Environment, the Regional EEM Coordinator, and scientists from Environment Canada (Appendix 1).

The EEM Coordinator is the primary contact for the mills and consultants on regional issues related to the EEM program. The primary role of the EEM Coordinator is to administer the EEM program and its related requirements under the PPER including the following:

- review of study designs, reports and interpretation of results,
- conduct of field audits and audit of sublethal toxicity test reports,
- communicate with the mills, consultants, TAP, scientists from other government departments and academia,
- advise the RAO of the adequacies of the study designs, site specific requirements, the quality of interpretive reports and the requirement for subsequent EEM studies, and
- ensure that all regional issues/problems are brought to the National level, so that EEM program is implemented consistently across Canada.

Table 1. Monitoring Conducted at Atlantic Mills for Cycle 2 EEM

Mill Name (Mill Identification Number)	Location	Fish Survey	Chlorinated Dioxin & Furan in Fish Tissue	Tracer Analysis	Fish Tainting Study	Benthic Invertebrate Community Survey	Effluent Sublethal Toxicity Test
Freshwater Mills:							
Abitibi-Consolidated Grand Falls (PP1007)	Grand Falls, NF	N	N	N	N	Y	Y
Fraser (PP1161)	Edmundston, NB	R	N	N	N	Y	Y
St. Anne-Nackawic (PP1144)	Nackawic, NB	Y	N	Y	N	Y	Y
Estuarine/Marine Mills:							
Abitibi-Consolidated Stephenville (PP1139)	Stephenville, NF	N	N	N	N	Y	Y
AV Cell (PP1112)	Atholville, NB	Y	N	Y	N	Y	Y
Bowater Maritimes (PP1113)	Dalhousie, NB	N	N	N	N	Y	Y
Bowater Mersey (PP1141)	Liverpool, NS	Y	N	Y	N	Y	Y
CKF/Minas Basin (PP1906)	Hantsport, NS	N	N	N	N	Y	Y
Corner Brook Pulp and Paper (PP1107)	Corner Brook, NF	N	N	N	N	Y	Y
Irving Paper (PP1074)	Saint John, NB	R	N	N	N	Y	Y
Irving Pulp & Paper (PP1143)	Saint John, NB	R	N	N	N	Y	Y
Kimberly-Clark NS (PP1160)	New Glasgow, NS	R	N	N	N	Y	Y
L.P. East River (PP1063)	Chester, NS	N	N	N	N	Y	Y
Lake Utopia (PP1159)	St. George, NB	N	N	N	N	Y	Y
Smurfit Stone Container (PP1111)	Bathurst, NB	Y	N	Y	N	Y	Y
Stora Enso (PP1152)	Port Hawkesbury, NS	Y	N	Y	N	Y	Y
UPM-Kymmene Groundwood (PP1081)	Miramichi, NB	N	N	N	N	Y	Y
UPM-Kymmene Kraft (PP1142)	Miramichi, NB	Y&R	N	Y	N	Y	Y

Y=Yes N=No R=Research Project

2.0 PULP AND PAPER INDUSTRY IN THE ATLANTIC REGION

There are 19 pulp and paper mills in the Atlantic Region that were subjected to the PPER during Cycle 2 (Figure 1). CKF and Minas Basin discharge effluent in close proximity to each other. As such, they are treated as one mill for the purpose of EEM, and have conducted a joint study for Cycle 2 as CKF/Minas Basin. Sixteen of the 19 mills discharge into an estuarine or marine environment, while three mills in the region discharge into a freshwater environment. There were 18 mills operating during Cycle 1 EEM and the first portion of Cycle 2; however, AV Cell Inc., re-commenced operations in 1998, and was required to conduct EEM for the remainder of Cycle 2.

The following mills have changed ownership since 1996 and are identified by their new names in the report: L.P. East River, formerly ABT(Canada) Ltd.; UPM-Kymmene, formerly REPAP; and Bowater Maritimes, formerly Avenor Maritimes.

The mill process, furnish, effluent treatment system and product produced for each mill in the Atlantic Region are presented in Table 2 and Table 3.

2.1 Mill Processes and Furnish

Pulp and paper mills use a variety of manufacturing processes and furnish to make many different types of pulp and paper products. In the Atlantic Region, nine mills use thermal mechanical pulping, four use kraft pulping, two use groundwood pulping and four use some form of sulfite process. Furnishes used in Cycle 2 include softwood, hardwood, kraft pulp, pulp, chemical pulp, recycled fibre, paper and boxes, and/or combination of these (Tables 2 and 3).

2.2 Use of Chlorine

Six mills in the Atlantic Region use Elemental Chlorine Free (ECF) bleaching process (Kimberly-Clark NS, St. Anne-Nackawic, UPM-Kymmene Kraft, Irving Pulp and Paper, Fraser and AV Cell). One mill, Stora Enso, uses peroxide (some peroxide is also used at Irving Pulp and Paper), and the 12 remaining mills do not use bleaching in their current operations.

2.3 BOD and TSS

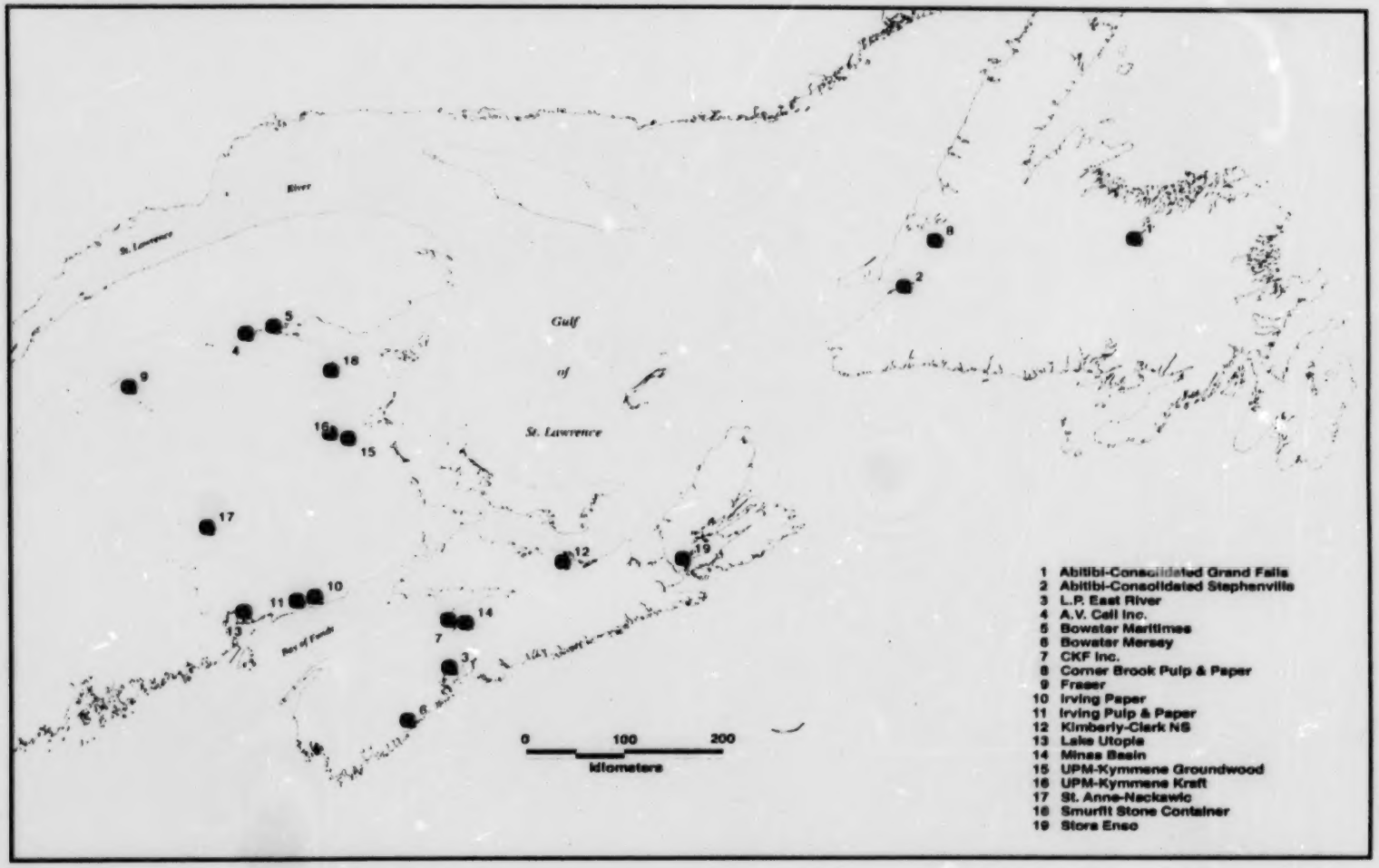
Many mills have made changes to their mill processes and effluent treatment since Cycle 1. These changes are reflected in the effluent quality data, which has shown major improvements in recent years. The BOD and TSS loadings at most of the mills have decreased since Cycle 1, and most mills are generally in compliance for the regulated parameters (personal communication with MT Grant, EC, March 2001).

2.4 Effluent Treatment

At present, 17 of the 19 mills utilize some form of secondary treatment, compared to only eight mills in Cycle 1 (Figure 2):

- eight mills currently use aeration basins,

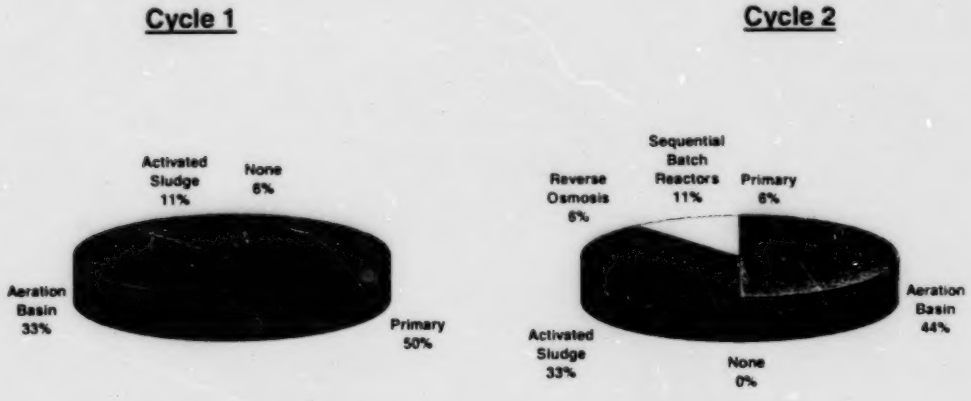
Figure 1. Pulp and Paper Mills in the Atlantic Region



- seven use activated slud1ge treatment by itself or in combination with other treatment,
- two use sequencing batch reactors,
- one mill uses Oxygen Delignification and a Reverse Osmosis System, and
- one mill has only primary treatment (Table 3).

The installment of secondary effluent treatment systems and additional changes to the effluent treatment systems have dramatically reduced the BOD and TSS loading to the receiving environment, and greatly reduced toxicity of effluent to organisms tested.

Figure 2. Effluent Treatment Types



2.5 Extent of 1% Effluent Plume and Effluent Flow

The zone of effluent mixing is often determined by a plume delineation study. In EEM, a 1% (100:1 effluent dilution) zone of effluent mixing has been defined as the exposure area. The delineation of the zone of effluent mixing is necessary to ensure that sampling stations are located within the zone of effluent mixing, and reference areas are well beyond any potential zone of impact. The maximum extent of 1% effluent zone and the mean effluent flow for Cycle 2 are presented in Table 4. Four mills have 1% effluent zones that extend less than 250 m from the outfall, and the remaining mills have 1% effluent zones that extend greater than 250 m. The maximum extent of the 1% effluent zone varies depending on site specific factors. As currents, wind speed, and local physical and hydrological factors influence the 1% effluent mixing zone, the data should be used with caution. The effluent flow ranged from 3 000 to 91 000 m^3/day. There were three mills that discharged less than 10 000 m^3 of effluent/day, nine mills that discharged greater than10 000 m^3 and less than 50 000 m^3 of effluent/day, and six mills that discharged greater than 50 000 m^3 of effluent/day.

Table 2. Pulp and Paper Mills in Atlantic Canada that Discharge into A Freshwater Environment

Mills	Original Start Up Date	Mill Process	Furnish	Start of Secondary Treatment	Effluent Treatment	Product
Abitibi-Consolidated Grand Falls	1905	TMP	Softwood	1996	ASB	Standard Newsprint
Fraser	1918	Bisulphite, GW, Recycled	Softwood, secondary fibre	1971	ASB	Bleached sulfite pulp
St. Anne-Nackawic	1969	Kraft	Hardwood	1969	ASB	Pulp

Table 3. Pulp and Paper Mills in Atlantic Canada that Discharge into an Estuarine/Marine Environment

Mills	Original Start Up Date	Mill Process	Furnish	Start of Secondary Treatment	Effluent Treatment	Product
Abitibi-Consolidated Stephenville	Early 1970s	TMP	Softwood	1970	ASB	Standard Newsprint
AV Cell	1920	DGSP	Softwood, Hardwood	1995	OAST	Pulp (baled)
Bowater Maritimes	1930	TMP	Softwood, Kraft pulp	1995	OAST	Newsprint
Bowater Mersey	1929	TMP	Softwood	1995	ASB	Newsprint
CKF/ Minas Basin	1933 1927	Liner board Pre-format	VCP, RF, GW pulp,	N/A	Primary – Screening + DAF	Paper Plates, Egg Cartons/ Liner board
Corner Brook Pulp and Paper	1925	TMP	Softwood, RP	1997	Activated Sludge + DAF	Newsprint
Irving Paper	1964	TMP	Softwood, hardwood, Kraft pulp bales	1995	ASB	Standard Newsprint, higher quality paper
Irving Pulp & Paper	1893	Kraft	Softwood, Hardwood	N/A	OD + RO	Kraft pulp bales/tissue
Kimberly-Clark NS	1967	Kraft	Softwood	1972	ASB	Bleached kraft pulp
L.P. East River	1967	TMP	Hardwood, Softwood	1970 increased aeration/ treatment in 1996	Activated Sludge + DAF	Hardboard
Lake Utopia	1971	NSSCP	Hardwood, RB	1994	Primary Clarification UASB, AST, Secondary Clarifier	Semi-chemical pulp and unbleached corrugating medium
Smurfit Stone Container	1914	NSSCP	Hardwood, RF	1995	UASB reactors followed by SBR Activated Sludge	Corrugating medium
Stora Enso	1961	TMP	Softwood, Hardwood	1995	Anaerobic with AST Polish	Newsprint, & super calendar paper
UPM-Kymmene Groundwood	1962	GW	Softwood	1995	SBR	Pulp (baled)
UPM-Kymmene Kraft	1948	Kraft	Softwood, Hardwood, pulp	Prior to 1992	ASB	Bleached kraft pulp and coated paper

NSSCP=Neutral Sulphite Semi-Chemical Pulp
TMP=Thermo-Mechanical Process
RO=Reverse Osmosis
OAST=Oxygen Activated Sludge Treatment
UASB=Upflow Anaerobic Sludge Blanket
DAF=Dissolved Air Floatation
RB=recycled boxes
VCP=virgin chemical pulp
DGSP=Dissolving Grade Sulphite Pulping
SBR=Sequencing Batch Reactors
ASB=Aeration Stabilization Basin
OD=Oxygen Delignification
AST=Activated Sludge Treatment
RF=recycled fibre
RP=recycled paper
GW=groundwood

Table 4. Pulp and Paper Mills in the Atlantic Region - Mean Cycle 2 Effluent Flow, Maximum Extent of 1% Plume, BOD and TSS

Mills	Discharge Environment	Mean Effluent Flow (m^3/day)	Maximum Extent of 1% effluent zone	BOD (t/day)	TSS (t/day)
Freshwater:					
Abitibi-Consolidated Grand Falls	Exploits River, NF	15,900	~75m (Cycle 1)	1.1	3.7
Fraser	Saint John River, NB	77,600	6.9Km (1999)	5.5	8
St. Anne-Nackawic	Saint John River, NB	65,900	~2.5km	1.9	1.8
Estuarine/Marine:					
Abitibi-Consolidated Stephenville	Bay St. George, NF	21,000	From 150 m to >4 km depending on wind and current speed	1.7	4.3
AV Cell	Restigouche, NB	48,000 to 50,000	1.7 km upstream, 3.3 km downstream	2-139 (1998), 19-79 (1999)	6.8-161 (1998), 36-152 (1999)
Bowater Mersey	Liverpool Bay, NS	27,700	Approx. 1 km	1.4	4.9
Bowater Maritimes	Dalhousie Harbour, NB	40,000	Ebb Tide: 250 to 350 m downstream, Slack Tide: 100 m outward to the estuary; Flood Tide: 350 m upstream	2.6	1.3
CKF/Minas Basin	Avon River, NS	7, 200 (combined)	<100 m	.37	.67
Corner Brook Pulp and Paper	Humber Arm, NF	80,400	Falling tide: 425 m Rising tide: 350 m.	4.15	5.2
Irving Paper	Courtenay Bay, NB	29,300 to 32,800	Not known	1.9-2.1	10.4-11.6
Irving Pulp & Paper	Saint John River, NB	91,300	5.3 km (high tide)	6.5	3.14
Kimberly-Clark NS	Northumberland Strait, NS	80,000	Approx. 2 km (model); Approx. 1 km (dye)	1.3	1.5
L.P. East River	Rous cove, NS	3,000	High Tide: 200 m, Low Tide: all measurements < 0.6%	0.3	0.7
Lake Utopia	L'Etang, NB	9,800	Approx. 4 km	0.7	1.1
Smurfit Stone Container	Bathurst Harbour, NB	14,700	Approx. 2 km	1.1	1.9
Stora Enso	Strait of Canso, NS	51,600	High tide: 100-250 m Low tide: 450 m	1.1	2.5
UPM-Kymmene Groundwood	Miramichi, NB	9,500	<250 m (intermittent discharge)	0.2	0.55
UPM-Kymmene Kraft	Miramichi, NB	75,300	Up to 325 m	3.4	8.3

3.0 FISH SURVEY

The review of the Cycle 1 data by the Working Group (Environment Canada, 1998) indicated that gonad weight, fecundity (reported as the total number of eggs/female) and liver weight are key parameters for studying effects of effluent on fish. At that time, the group recommended that, in the absence of strong guidance on what constitutes an ecologically significant effect, a 25% difference in gonad weight between the reference and exposed fish could be set as a target effect size to help determine whether impacts were occurring or not. Since the Cycle 1 review, expanded data interpretation guidance, including a definition of an effect on the fish population and how EEM results may be interpreted in terms of "Impact or No Impact" has been developed. For the purposes of Cycle 2 evaluation, the definition of effect has been defined as a 'statistically significant difference measured in an environmental variable between the mill effluent exposure area and a reference area' (Environment Canada, 1998).

In general, a standard fish survey should have been conducted in Cycle 2 if (Courtenay *et al.*, 1998):

- 'sufficient number' of species that have the 'suitable characteristics' of sentinel species were found in Cycle 1 (Environment Canada, 1998), or
- the fish survey study design could be modified using different capture technique, timing, sentinel species selection, or reference site choice, and
- if there are no confounding factors associated with other effluent discharges or historical damage.

However, due to an insufficient number of fish caught in Cycle 1, inability to confirm effluent exposure and duration of exposure (Parker and Smith, 1999), only 6 mills attempted a fish survey in Cycle 2 in the Atlantic Region with 1 mill not capturing enough fish to conduct an assessment.

3.1 Objective

The primary objective of the fish survey is to evaluate the effects of effluent on the survival, growth and reproduction of fish (Environment Canada, 1997). The following fish parameters were assessed as required by Annex 1 of PPER: age, size at age, condition, gonad weight, fecundity, and liver weight.

3.2 Results and Discussion

Only 33% (6 of 18) of the EEM studies conducted a fish survey in Cycle 2 (1 freshwater mill and 5 estuarine/marine mills). Of these six mills, one mill did not capture enough fish to complete the assessment. The remaining 67% (12 of 18) of the mills did not include a fish study due to:

- rapid dilution - 1% effluent zone being less than 250 metres, or
- insufficient number of resident fish and/or species, or
- alternative studies (research) being conducted.

The lack of knowledge on local fisheries, fish movement and migration patterns, in addition to lack of alternative methods to assess effects of effluent on fish were key factors in the low number of fish studies conducted in Cycle 2 in the Atlantic Region.

Five mills (28%) participated in studies to explore alternative methods for assessing effects of their effluent on fish. The alternative methods included fish life-cycle mesocosm and caged bivalve studies.

3.2.1 Sentinel Species Used

Annex 1 of the Aquatic Environmental Effects Monitoring Requirements (Environment Canada, 1997) requires a mill to select two sentinel species for the fish survey. The number of sentinel species selected was reduced from 13 species in Cycle 1 to five in Cycle 2 (Table 5), due to difficulties in catching sufficient number of fish. Three studies targeted two sentinel species, but only two were successful (Smurfit Stone Container and AV Cell).

Table 5. Fish Species used in Cycle 2

Species	# of Studies used
Mummichogs (*Fundulus heteroclitus*)	3
Winter flounder (*Pleuronectes americanus*)	1
Three-spine stickleback (*Gasterosteus aculeatus*)	2
Yellow perch (*Perca flavescens*)	1
Longhorn sculpins (*Myoxocephalus octodecemspinosus*)	2

Table 6 lists the sentinel species selected by each mill. The most commonly used species was mummichogs (*Fundulus heteroclitus*), which was used in three of the six field studies, and in mesocosm studies at three mills in New Brunswick: Irving Paper, Irving Pulp and Paper, and UPM-Kymmene Kraft. In addition, a caged bivalve pilot study using blue mussels (*M. edulis)* was conducted at Kimberly-Clark NS, and a fathead minnow (*Pimephales promelas*) life-cycle bioassay, and a caged fish study using white suckers (*Catostomus commersoni*) were conducted at Fraser.

3.2.2 Sample Size

Sample size depends on the magnitude of the effect one wants to detect and the variability in the endpoint to be measured. As such, the number of samples required for each endpoint differed for each mill.

3.2.3 Capture Methods

A wide array of capture methods was used by the mills in Cycle 2 (Table 7). Smurfit Stone Container, UPM-Kymmene Kraft and AV Cell used baited minnow traps and beach seine nets, while St. Anne-Nackawic used angling methods due to fisheries restrictions. At the Bowater Mersey mill, the consultants used both baited long-lines and gillnets, but were unsuccessful in capturing sufficient numbers of the targeted sentinel species. The success of these methods are based on calculation of catch per unit effort (CPUE), and expressed as fish caught/hour, or some other similar form in Table 7. In general, the level of effort ranged from two to six days in Cycle 2, which is similar to Cycle 1.

3.2.4 Tracers

Two mills used resin acids as a tracer of exposure in fish. One study showed greater exposure to effluent in the near-field (NF) than the reference, but the other study was less clear. The use of resin acids as a tracer in Cycle 2 was not very effective due to limited sample size. In addition, carbon:nitrogen (C:N) stable isotope was used at St. Anne-Nackawic, but due to the small sample size, the results were inconclusive. Other mills used water quality measurements as a tracer to determine the zone of exposure.

3.2.5 Tissue Analysis: Chlorinated Dioxin and Furan Congeners

In 1992, regulations under the *Canadian Environmental Protection Act* (CEPA) introduced limits on the dioxin/furan levels in effluent. Pulp mills that use or have used bleaching were required to analyze for dioxin/furan congeners in tissue on edible portions of fish unless the mill could show that:

"Levels of dioxins in the previous cycle were below the consumption guidelines or there is no consumption guideline-related advisory/closure based on dioxin/furan contamination for any species or tissue in the receiving environment of the mill

and;

The mill has been in compliance with the CEPA regulations for dioxins and furans in final mill effluent for the preceding 12 months." (Environment Canada, 1998)

Majority of the mills (12 out of 19) in the Atlantic Region does not use chlorine bleaching in their current operation and are not subjected to the dioxin and furan requirement in fish tissue. In Cycle 2, none of the pulp and paper mills in the Atlantic Region using bleaching process were required to conduct the dioxin/furan analysis in tissue because the mills demonstrated the above.

Table 6. Sentinel Species Used in Cycle 1 and Cycle 2

Mills	Cycle 1	Cycle 2
Abitibi-Consolidated Grand Falls	• Threespine Stickleback (*Gasterosteus aculeatus*)	N/A: 1% plume <250 m
Abitibi-Consolidated Stephenville	• Lobster (*Homarus americanus*) • Winter Flounder (*Pleuronectes americanus*)	N/A: buoyant surface plume, rapid dilution and confounding discharge from Port Harmon.
AV Cell	Began operation after Cycle 1	• Threespine Stickleback (*Gasterosteus aculeatus*) • Mummichog (*Fundulus heteroclitus*)
Bowater Maritimes	• Rock Crab (*Cancer irroratus*) • Winter Flounder (*Pleuronectes americanus*)	N/A: lack of sentinel species at the site
Bowater Mersey	• Longhorn Sculpin (*Myoxocephalus octodecemspinous*) • Winter Flounder (*Pleuronectes americanus*)	• Longhorn Sculpins – Could not catch enough to perform an AFS (*Myoxocephalus octodecemspinous*)
CKF/Minas Basin	• Atlantic Silverside (*Menidia menidia*) • Atlantic Tomcod (*Microgadus tomcod*)	N/A: 1% plume <250 m
Corner Brook Pulp and Paper	• Cunner (*Tautogolabus adspersus*) • Winter Flounder (*Pleuronectes americanus*)	N/A: Fish Study Not Done – lack of suitable sentinel species and confounding factors
Fraser	• White Sucker (*Catostomus commersoni*) • Yellow Perch (*Perca flavescens*)	N/A: Research – Fathead Minnow Life Cycle Test (*Pimephales promelas*), wild fish caged study using white suckers (*Catostomous commersoni*) and slimy sculpin (*Cottus cognatus*).
Irving Paper	• Atlantic Tomcod (*Microgadus tomcod*) • Green Crab (*Carcinus maenas*)	N/A: Research – Mesocosm Study using Mummichogs (*Fundulus heteroclitus*)
Irving Pulp & Paper	• Atlantic Tomcod (*Microgadus tomcod*) • Green Crab (*Carcinus maenas*) • Mummichogs (*Fundulus heteroclitus*)	N/A: Research – Mesocosm Study using Mummichogs (*Fundulus heteroclitus*)
Kimberly-Clark NS	• Rock Crab (*Cancer irroratus*) • Winter Flounder (Pleuronectes americanus)	N/A: Research – Caged Bivalve Study (*Mytilus edulis*)

Table 6. Sentinel Species Used in Cycle 1 and Cycle 2 (continued)

Mills	Cycle 1	Cycle 2
L.P. East River	• Longhorn Sculpin (*Myoxocephalus octodecemspinous*) • Winter Flounder (*Pleuronectes americanus*)	N/A: 1% plume <250 m
Lake Utopia	• Atlantic Tomcod (*Microgadus tomcod*) • Green Crab (*Carcinus maenas*)	N/A: Fish Reconnaissance Survey indicated no suitable species
Smurfit Stone Container	• Atlantic Tomcod (*Microgadus tomcod*) • Winter Flounder (*Pleuronectes americanus*)	• Threespine Stickleback (*Gasterosteus aculeatus*) • Mummichog (*Fundulus heteroclitus*)
St. Anne-Nackawic	• White Sucker (*Catostomus commersoni*) • Yellow Perch (*Perca flavescens*)	• Yellow Perch (*Perca flavescens*)
Stora Enso	• Rock Crab (*Cancer irroratus*) • Winter Flounder (*Pleuronectes americanus*)	• Winter Flounder (*Pleuronectes americanus*) • Longhorn Sculpin (*Myoxocephalus octodecemspinous*)
UPM-Kymmene Groundwood	• Atlantic Tomcod (*Microgadus tomcod*)	N/A: 1% plume <250 m
UPM-Kymmene Kraft	• Atlantic Tomcod (*Microgadus tomcod*) • White Sucker (*Catostomus commersoni*)	• Mummichog • Also conducted research – Mesocosm Study using Mummichogs (*Fundulus heteroclitus*)

Table 7. Fishing Methodology and Catch Per Unit Effort for Cycle 2

Mills	Date	Method	CPUE	Cycle 2 Sentinel Species	Total Fish Caught
AV Cell	May 25&29, 1999	Beach seines, minnow traps	7 mummichogs/hour, 6 sticklebacks/hour	Mummichog, Threespine Stickleback	**Mummichog:** E: 25F, 25M R: 26F, 20M **Stickleback:** E: 20F, 22M R: 21F, 21M
Bowater Mersey	Oct. 20-24, 1998	Gillnets and baited long lines	R: 0.06 fish/1000 foot hours, E: 0.00 fish/1000 foot hours	N/A	3
Smurfit Stone Container	May 17-19, 1999	Beach seines, minnow traps	R1: S=5.73 fish/m^2, M=7.98 fish/m^2, R2: S=2.22 fish/m^2, M=0.60 fish/m^2, E: S=0.10 fish/m^2, M=0.05 fish/m^2	Mummichog, Threespine Stickleback	**Mummichog:** E: 20F, 20M. 56-NP R1: 25F, 25M, 429-NP R2: 20F, 20M, 72- NP **Stickleback:** E: 20F, 20M. 233-NP R1: 25F, 25M, 304-NP R2: 20F, 20M, 266-NP
St. Anne-Nackawic	Oct. 11-15, 1999	Angling	R:1.67 fish/angler hour, E: 0.94 fish/angler hour	Yellow Perch	E: 29F, 13M, 6I R: 8F, 20M, 9I, 6-NP
Stora Enso	Sept. 4-9, 1999	Gillnets	E: 2.82 fish/1000 foot hours, R:1.02 fish/1000 foot hours	Winter Flounder & Longhorn Sculpin	**Winter flounder:** E: 41F, 31M R: 40F, 29M
UPM-Kymmene Kraft	May 17-21, 1999	baited minnow traps & beach seine nets	8.6 fish/hour	Mummichog	E: 26F, 35M, 1I R: 22F, 25M

E=Exposure area R=Reference area NP=Not Processed I=Immature
S=Beach Seine M=Minnow Traps

3.2.6 Summary of Fish Studies

The most common effects of pulp and paper mill effluent on fish that were documented prior to Cycle 2 were (Munkittrick *et al.*, 1994; Sandstrom *et al.*, 1988, Sandstrom 1996):

- increased condition factor
- delayed sexual maturation
- decreased gonad size
- increased liver size

The results of the fish studies conducted in Cycle 2 are presented in Table 8. Some of the effects observed in Cycle 1 were again observed in Cycle 2 at the same mill. A combination of the following differences was observed between effluent exposed and reference fish:

- increased condition in fish from exposure area
- increased liver size in fish from exposure area
- decreased liver size in fish from exposure area
- decreased gonad weight in fish from exposure area
- decreased egg number in fish from exposure area

In general, the differences found were not consistent between both sexes of the same fish species, between species when two species were assessed in the same study, and between mills in the Atlantic Region.

The results from the St. Anne-Nackawic study are similar for both Cycles 1 and 2. A decrease in growth rate, gonad and liver weights were observed in the exposed yellow perch in both cycles. Since there are other sources of nutrients and other contaminants in the exposure area, and as the reference area is questionable, it is difficult to solely attribute the observed effects to the pulp mill effluent. The fish from the reference area may be growing faster and have heavier livers due to nutrient enhancement.

The AV Cell study used three-spine sticklebacks and mummichogs. For sticklebacks, females were predominant in the exposure area. A decrease in condition, liver weight and smaller eggs were observed in the exposed female sticklebacks. An increase in liver weight was observed for the exposed male sticklebacks. In addition, the small females had smaller ovaries.

For mummichogs, the females in the exposure area were slightly younger and heavier than females from the reference area. 21% (weight standarized) – 29% (length standarized) more eggs were present in a 'standarized' fish from the exposure than the reference area. The males from the exposure area were older, heavier and longer than males from the reference area. In addition, the testis weight increased more rapidly with increasing body length in the exposure area.

Inconsistent results were observed with sticklebacks and mummichogs. As the duration of the exposure of fish to effluent is unknown, the observed effects could be due to different species sensitivity and/or that the sticklebacks had higher/longer exposure to the effluent than mummichogs.

At UPM-Kymmene Kraft, a decrease in the number of eggs per exposed female mummichog was observed.

At Smurfit Stone Container, two species were assessed - mummichog and three-spine stickleback, and two reference sites were used. A decrease in age, and increase in liver weight were found in exposed female mummichogs and both sexes of sticklebacks. As tracers were not used to confirm exposure, the duration of exposure of fish to effluent is questionable. In addition, the fish from the reference area may not be representative of the fish in that area. The fish from the reference area were all collected from a small area over a short period of time, while the exposure fish were collected at a series of locations over a number of days. It is possible that this resulted in the random capture of schools in the reference and exposure areas dominated by different age classes (Courtenay, in preparation).

At Stora Enso, only winter flounders were caught in sufficient number for analysis. The exposed flounders had decreased size at age and males had increased liver size. These results are consistent with what was observed in Cycle 1. Even though effects were found in the exposure area, it is difficult to solely attribute these effects to the discharge of pulp mill effluent, as there are other industrial discharges nearby, and deposits of wood waste from historical operation are present in the exposure area. Because winter flounders are benthic, these deposits may affect the fish's food source. Reproductive endpoints were not measured, as the flounders were immature.

At Bowater Mersey, four days of fishing effort resulted in three fish. The fish survey was not completed at this site.

Table 8. Results of Cycle 2 EEM Fish Studies in Atlantic Region

Mills	Cycle 1 Sentinel Species	Cycle 1 Effects in Exposure Area	Cycle 2 Sentinel Species	Cycle 2 Effects in Exposure Area
Freshwater:				
Abitibi-Consolidated Grand Falls	Threespine Stickleback	F: shorter		N/A - 1% plume <250 m
Fraser	White Sucker, Yellow Perch	**White Sucker:** F: longer and heavier, decreased fecundity M: reduced gonad size **Yellow Perch:** M&F: lower fork length F: decreased fecundity	Fathead Minnow	**Research :** 125-day Fathead Minnow Life Cycle Test – • At 3% effluent exposure, male fish had the appearance of ovipositors. • At 30-100% effluent concentration, the majority of males looked externally like females. • At 4-month effluent exposure, a change in external sex characteristics observed. • At 1% effluent exposure, some females had male sex characteristics. • Increase in growth seen in as little as 30 days. (Section 6.4)
St. Anne-Nackawic	White Sucker, Yellow Perch	**White Sucker:** M&F: older, decreased growth rate, lower total weight and liver weights F: reduced reproduction capacity **Yellow Perch:** M: older and lower liver and overall weight F: reduced reproductive capacity M&F: decreased growth rate	Yellow Perch	M: decrease in length, gonad weight and liver weight F: decrease in length, 40% decrease in weight, smaller gonads & eggs, decrease in liver weight
Estuarine/ Marine:				
Abitibi-Consolidated Stephenville	Lobster, Winter Flounder	**Lobster:** F: longer M: large body & gonad weight		N/A - rapid dilution
AV Cell	Began operations during Cycle 2	No EEM study, mill opened in 1998	Threespine-Stickleback, Mummichog	**Sticklebacks:** More females at the exposure area M: increase in liver weight, F: decrease in gonad and total weight, smaller eggs **Mummichogs:** M: older, higher condition F: slightly younger, heavier, 21-29% more eggs in a 'standarized' fish from exposure area (see text).

Table 8. Results of Cycle 2 EEM Fish Studies in Atlantic Region (continued)

Mills	Cycle 1 Sentinel Species	Cycle 1 Effects in Exposure Area	Cycle 2 Sentinel Species	Cycle 2 Effects in Exposure Area
Bowater Maritimes	Rock Crab, Winter Flounder	**Winter Flounder:** F: greater growth rates in future outfall area **Rock Crabs:** M: larger gonad weight		N/A – reconnaissance survey
Bowater Mersey	Longhorn Sculpin, Winter Flounder	**Longhorn Sculpin:** F: younger, gonad weight higher M&F: liver weight higher		Could not catch enough fish to perform an AFS
CKF/Minas Basin	Atlantic Silverside, Atlantic Tomcod	**Atlantic Tomcod:** M&F: higher liver weight F: reduced gonad weight		N/A - 1% plume <250 m
Corner Brook Pulp and Paper	Cunner, Winter Flounder	**Winter Flounder:** F: growth rate high **Cunners:** M: larger gonads M&F: larger liver, longer and heavier		Fish Study Not Done – Confounding Factors
Irving Paper	Atlantic Tomcod, Green Crab	**Atlantic Tomcod:** F: heavier liver, gonad, overall weight	Mummichogs	**Research – Mesocosm Study using mummichogs** • 28-day 3% Effluent exposure • Significantly reduced gonad weight in both sexes • Significant increase in DHAA found in bile of exposed males • liver weight unaffected
Irving Pulp & Paper	Atlantic Tomcod, Green Crab	**Atlantic Tomcod:** M: lower weight F: lower gonad weight	Mummichogs	**Research – Mesocosm Study using Mummichogs** • 30-day and 60-day exposure to 5% effluent • After 30 days, liver size significantly increased in exposed adult females • After 60 days, liver size significantly increased among exposed adults of both sexes • After 60 days, total body weight and length of juveniles significantly decreased
Kimberly-Clark NS	Rock Crab, Winter Flounder	**Winter Flounder:** F: younger **Rock Crab:** M: smaller carapace width	Blue Mussels	**Research – Caged Bivalve Study** • No effect; however, increased growth observed at the site closest to the outfall.
L.P. East River	Longhorn Sculpin, Winter Flounder	**Longhorn Sculpin:** F: older M: higher gonad weight,higher Growth rate; M&F: heavier and longer, higher liver weight;		N/A - 1% < 250 m

Table 8. Results of Cycle 2 EEM Fish Studies in Atlantic Region (continued)

Mills	Cycle 1 Sentinel Species	Cycle 1 Effects in Exposure Area	Cycle 2 Sentinel Species	Cycle 2 Effects in Exposure Area
Lake Utopia	Atlantic Tomcod, Green Crab	**Green Crab:** M: hepatopancreas higher weight		N/A - Fish Reconnaissance Survey
Smurfit Stone Container	Atlantic Tomcod, Winter Flounder	**Tomcod:** M: reduced gonad weight **Winter Flounder:** F: reduced liver weight	Threespine-Stickleback, Mummichog	**Sticklebacks:** M: decrease in age and body weight; increase in liver weight F: decrease in age, body weight and gonad weight; increase in liver weight **Mummichogs:** M: decrease in age, F: decrease in age; increase in liver weight
Stora Enso	Rock Crab, Winter Flounder	**Winter Flounder:** F: lower length, overall weight, larger liver M: younger, lower length, testis size M&F: decreased growth rate **Rock Crab:** M&F: decreased growth rate M: lower carapace width, increased hepatopancreas and gonad growth rate F: lower overall weight, higher hepatopancreas weight	Winter Flounder, Longhorn Sculpin	**Winter Flounder:** M: body slightly smaller in size, larger livers F: younger (only 2 sexually mature female flounders were caught)
UPM-Kymmene Groundwood	Atlantic Tomcod	F: egg volume lower		N/A - 1% plume <250 m
UPM-Kymmene Kraft	Atlantic Tomcod, White Sucker	**Atlantic Tomcod:** M&F: heavier gonad and liver weight	Mummichogs	A decrease in the number of eggs in exposed female. **Research – Mesocosm Study** • 5 fold decrease in testosterone levels

3.2.7 Problems and Issues in Cycle 2 Fish Studies

In general, there was greater success in the fish monitoring in Cycle 2 than in Cycle 1. However, some of the problems encountered in Cycle 1 were again found in Cycle 2, such as the unknown degree of exposure of fish to effluent (duration of exposure), other effluent discharges nearby, insufficient number of fish being caught, and DFO fisheries restrictions such as fishing window and fishing gear.

4.0 INVERTEBRATE COMMUNITY SURVEY (ICS)

The potential effects of pulp mill effluent on benthic communities include changes in abundance, richness and diversity. Generally, a reduction in the number of taxa (diversity) and an increase in abundance of certain taxa have been associated with "disturbed" benthic communities. Environmental (physical, chemical and/or biological) stress can inhibit the survival, growth and reproduction of intolerant taxa, and increase the abundance of the most tolerant taxa.

EEM defines an effect as "a statistically significant difference measured in an environmental variable between the mill effluent exposure area and a reference area" (Environment Canada, 1998).

4.1 Objective

The objectives of the benthic invertebrate community survey are to delineate the spatial extent and magnitude of habitat degradation due to organic enrichment or other forms of contamination due to pulp mill effluent. The goal of ICS is to determine the structural differences (abundance, number of taxa, shifts in the kinds and dominance of taxa) in invertebrate communities between the exposed and reference areas (Environment Canada, 1998).

4.2 Results and Discussion

4.2.1 Study Designs

All mills in the Atlantic Region conducted an invertebrate community survey. The details of the study designs selected by the mills are presented in Table 9. Various designs including control/impact, multiple control/impact, and single gradient and multiple gradient designs were used in Cycle 2. Seven studies (39%) used the gradient design, eight studies (43%) used the control/impact design, and three mills (17%) used a combination of control impact and gradient designs (Table 9).

Some mills used more than one design/approach to assess effects of their effluent on benthic communities. Smurfit Stone Container used both control/impact and gradient designs. Kimberly-Clark NS and L.P. East River conducted a control/impact benthic study and a gradient intertidal survey. Fraser used D-shaped nets and artificial substrate samplers to assess the effects of its effluent on the benthic community. Corner Brook used artificial substrate samplers (ASS) to differentiate the effects of current effluent discharges from historical wood fibre deposits.

The majority of the mills (83%) studied depositional habitat, while three mills (17%) examined erosional and/or intertidal habitats. In general, the selected study designs provided useful information on the effects of effluent on the benthic community. In many cases, however, the results were confounded by other effluent inputs in the area and/or historical deposit of wood waste.

Table 9. Cycle 2 EEM Benthic IC Study Designs in the Atlantic Region

Mills	Study Design Selected	Habitats Sampled	Sampling Device	Sieve Size	# of Reference Areas	# of Exposure Areas	# of Reference Stations/ Habitat	# of Exposure Stations/ Habitat	# of Stations/ Gradient	# of Samples/ Station
Freshwater										
Abitibi-Consolidated Grand Falls	Multiple Control/Impact	Erosional	D-shaped net	500 µm	3	1	5	5	N/A	2
Fraser	Multiple Control/Impact	Erosional/AS	Surber Sampler	500 µm	3	2	5	5	N/A	3
			AS	500 µm	1	1	3	3	N/A	3
St. Anne-Nackawic	Multiple Control/Impact	Depositional	Shallow: Standard Ponar Grab (0.052 m^2), Deep: weighted Ekman Grab (0.023 m^2)	500 µm	2(1-shallow, 1-deep)	3(1-shallow, 2-deep)	5	5	N/A	3/Ponar; 5/Ekman
Estuarine/Marine										
Abitibi-Consolidated Stephenville	Multiple Gradient	Depositional	Van Veen Grab (0.07 m^2)	500 µm	N/A	N/A	N/A	N/A	20 stations total: 6 North of outfall, 14 South of outfall	2
AV Cell	Multiple Gradient - up and downstream	Depositional	Petite Ponar Grab (0.023 m^2)	500 µm	1	N/A	3	N/A	7 d/s and 5 u/s	3
Bowater Maritimes	Multiple Control/Impact	Depositional	Standard Ponar Grab (0.052 m^2)	500 µm	2	1	5	5	N/A	2
Bowater Mersey	Control/Impact	Depositional	Petite Ponar Grab (0.023 m^2)	500 µm	1	3	5	5	N/A	5

Table 9. Cycle 2 EEM Benthic IC Study Designs in the Atlantic Region (continued)

Mills	Study Design Selected	Habitats Sampled	Sampling Device	Sieve Size	# of Reference Areas	# of Exposure Areas	# of Reference Stations/ Habitat	# of Exposure Stations/ Habitat	# of Stations/ Gradient	# of Samples/ Station
CKF/Minas Basin	Multiple Gradient (2 transects)	Depostional	Van Veen Grab (0.12 m^2)	1mm	N/A	N/A	N/A	N/A	8	3
	Multiple Gradient (2 transects)	Intertidal	0.1 m^2 quadrats	N/A	N/A	N/A	N/A	N/A	8	3
Corner Brook Pulp and Paper	Multiple Control/Impact	Depositional	BASS samplers	1 mm	5	1NF, 1FF	5	5 NF, 10 FF	N/A	1
Irving Paper	Multiple Gradient (3 transects)	Depositional	Standard Ponar Grab (0.05 m^2)	1 mm	N/A	N/A	N/A	N/A	16 Total	5 @ 14 stations, 10 @ 2 stations
Irving Pulp & Paper	Simple Gradient	Depositional	Van Veen Grab (0.12 m^2)	1 mm	N/A	N/A	N/A	N/A	15	5 @13 stations, 10 @ 2 stations
Kimberly-Clark NS	Control/Impact	Depostional	Petite Ponar Grab (0.023 m^2)	500 µm	1	2 (NF, FF)	7	5	N/A	5
	Qualitative Intertidal Survey (6 transects)	Intertidal	0.5m X 0.5 m quadrat	N/A	N/A	N/A	N/A	N/A	3	1
L.P. East River	Multiple Control/Impact	Depositional	Van Veen Grab (0.1 m^2)	500 µm	5	2 (NF, FF)	5	5NF, 1FF	N/A	5
	Gradient – Intertidal	Intertidal (7 transects)	0.25m X 0.25m quadrats	N/A	N/A	N/A	N/A	N/A	5	1

Table 9. Cycle 2 EEM Benthic IC Study Designs in the Atlantic Region (continued)

Mills	Study Design Selected	Habitats Sampled	Sampling Device	Sieve Size	# of Reference Areas	# of Exposure Areas	# of Reference Stations/ Habitat	# of Exposure Stations/ Habitat	# of Stations/ Gradient	# of Samples/ Station
Lake Utopia	Impoundment – Gradient	Depositional	Impoundment: Eckman Grab (0.05 m^2)	500 µm	N/A	N/A	N/A	N/A	11	6
	Estuary – Gradient	Depositional	Estuary - Van Veen Grab (0.12 m^2)	1 mm	N/A	N/A	N/A	N/A	14	5
Smurfit Stone Container	Control/Impact	Depositional	Standard Ponar Grab (0.052 m^2)	500 µm	1	1	5	5	N/A	3 grabs composited into one sample
	Simple Gradient	Depostional	Standard Ponar Grab (0.052 m^2)	500 µm	N/A	N/A	N/A	N/A	30 (15 in <1% effluent, 15 in >1% effluent)	3 grabs composited into one sample
Stora Enso	Control/Impact	Depositional	Petite Ponar Grab (0.023 m^2)	500 µm	2	3	5	5NF, 2FF, 3/ONF	N/A	5
UPM-Kymmene Groundwood	Gradient	Depositional	Van Veen Grab (0.093 m^2)	500 µm	N/A	1	N/A	N/A	6 upstream gradient and 2 down stream	7/exposure station, 5/reference station
UPM-Kymmene Kraft	Multiple Control/Impact	Depositional	Van Veen Grab (0.093 m^2)	500 µm	2 (shallow, deep)	2(shallow, deep)	4	4	N/A	7/exposure station, 5/reference station

4.2.2 Methods

4.2.2.1 Sampling Period

Most of IC studies (72%) were conducted in the fall (September - October); 22% (4 of 18) in summer (July - August) and 6% during summer to fall. Corner Brook Pulp & Paper conducted an artificial substrate study, which deployed the buckets during summer and retrieved them in the fall. In addition to the traditional fall IC studies to assess temporal changes in benthic communities, Fraser also conducted an artificial substrate study that deployed buckets during summer and retrieved during fall.

4.2.2.2 Sampling Device

The following samplers were used to collect samples in Cycle 2:

- the Van Veen grab sampler was used by 39% of the mills (7 of 18)
- the Standard Ponar grab sampler was used by 22% of the mills (4 of 18)
- the Petite Ponar grab sampler was used by 22% of the mills (4 of 18), and
- Artificial Substrate Samplers (ASS), Ekman grab, D-shaped nets and Surber samplers were used at the remaining mills.

4.2.2.3 Mesh Size

28% (5 of 18) of studies used the 1mm mesh to sieve the sediments and the remaining 72% of the studies used the 500 µm sieve. The 1 mm sieve was exclusively used by the marine mills. The TGD (Environment Cananda, 1998) recommends 1 mm and 500 µm sieve sizes to be used for the mills that discharge into estuarine/marine systems. The 1 mm portion is to be processed, and the 500 µm portion is to be archived only to be used when necessary; however, in an attempt to compare results with Cycle 1, 500 µm sieve size was used at some of the marine mills. All the mills that discharge into freshwater used 500 µm sieve size.

4.2.2.4 Replicates (field sub-samples/station)

The number of replicate samples required to detect an effect of a given size is dependent on the homogeneity of the benthic community at the study and reference sites and on the levels of significance that are used (Environment Canada, 1998). The number of replicate samples required was determined by the variability observed in benthic data from Cycle 1 in most cases. The number of replicate samples ranged from 1 to 10 samples, with over 50% of the studies taking 5 samples/station (Table 9).

4.2.2.5 Laboratory Sub-Sampling

Laboratory sub-sampling occurred at most of the mills. The 'reported' sub-sampling error ranged from 0 to 18%. A complete summary of the sub-sampling information is presented in Table 10.

The following sub-sampling methods were used:

- a minimum of 100 organisms per sub-sample with 20% precision
- a minimum of ¼ fraction with 20% precision, and
- small fractions as long as the difference between 2 sub-samples was < 20%.

In most cases, the total number of organisms actually counted in a grab sample, prior to sub-sampling was not reported, and as such, the sub-sampling error for most of the mills is not known. Most of the studies reported the variability between 2 sub-samples and these are presented in Table 10.

4.2.2.6 Sorting Efficiency

Samples were re-sorted to determine the success of initial sorting on 10% of samples as recommended by TGD (Environment Canada, 1998) by all mills. The sorting efficiency ranged from 94-100% (Table 10).

4.2.3 Data Analysis

All mills calculated the total abundance and the number of taxa as recommended by the TGD (Environment Canada, 1998). In addition, the mills used a number of different analytical techniques to assess invertebrate communities and spatial patterns. These included cluster analysis (Bray-Curtis similarity coefficients, Non-metric Multidimensional Scaling [NMDS]), diversity indices (dominance index, richness index, Shannon-Weaver Index), functional feeding groups (filterers, shredders), indicator taxa (EPT index, pollution sensitive/tolerant organisms, shift in taxa), correspondence analysis, principal component analysis, dominance curves, geometric size classes, and others.

4.2.4 Summary of Benthic Invertebrate Community Studies

The results of Cycle 2 benthic invertebrate community studies are presented in Table 11.

As presented in Table 11, 33% of the studies (6 of 18) concluded that the mill's receiving area is not impaired as indicated by no statistical differences in abundance, richness and diversity indices:

- In three studies where statistical effects were not found in benthic indices (i.e., total abundance, richness and diversity), a difference in species composition was observed: Kimberly-Clark (Polychaete), Fraser (EPT) and Bowater Maritimes (Polychaete).

- In another study (CKF/Minas Basin), statistical difference was not found when number of taxa was regressed against distance from the outfall ($p=0.08$, power=0.412, $\alpha= 0.05$). Eventhough a statistical difference was not found, at one transect, the number of taxa decreased with the distance from outfall, suggesting nutrient enhancement; however, subsequent testing with TOC, sulfide and redox measurement did not support nutrient enrichment.

Table 10. Cycle 2 Benthic IC Study - Sub-sampling and Sorting QA/QC

Mills	Fraction Subsampled	Difference between 2 fractions (%)	Sub-Sampling Method	Sorting Efficiency (%)
Fresh Water				
Abitibi-Consolidated Grand Falls	NR	NR	NR	95.4
Fraser	NR	NR	Ontario Ministry of Environment and Energy and US EPA guidelines	95.7
St. Anne-Nackawic	whole grab – 1/4	8.57-16.18	BEL[1]	99.3
Estuarine/Marine				
Abitibi-Consolidated Stephenville	NR	NR	NR	100
AV Cell	whole	N/A	N/A	99.6-100
Bowater Maritimes	whole grab – 1/4	0.46-0.58	BEL	96.7
Bowater Mersey	whole grab – 1/8	1-15.5	N/R	95.3
CKF INC./Minas Basin	whole grab – 1/2	2.9	HSSS[2]	99.2
Corner Brook Pulp and Paper	whole grab – 1/4	0-4.0	BEL	99.4
Irving Paper	whole grab – 1/2	5.2	HSSS	100
Irving Pulp & Paper	NR	NR	HSSS	99
Kimberly-Clark NS	whole grab - 1/2	NR	BEL	95.3
L.P. East River	whole grab – 1/4	9.6-18.1	Matoda Plankton Splitter	98.9
Lake Utopia	whole grab - ½	Imp: 2.8 Est: 4.6	HSSS	Imp: 96.9 Est: 96.4
UPM-Kymmene Kraft	whole grab – 1/4	7.4-15.7	*	94.6-100
UPM-Kymmene Groundwood	whole grab – 1/4	7.4-15.7	*	93.8-100
Smurfit Stone Container	whole grab -1/32	2.9-7.0	BEL	98.3
Stora Enso	whole grab – 1/4	15.84	BEL	99.2

[1]Benthic Ecology Lab Technique (BEL): Whole sample is deposited into a 500 µm sieve and washed. The sample is drained and then weighed. Once weighed, an appropriate size subsample is removed and washed thoroughly to remove debris and remaining preservative.

[2]HSSS=Humboldt Sediment Sample Splitter

* After elutriation, the remaining debris and organisms are washed through a series of 2 sieves (3.36 mm and 500 µm). The material on 3.36 mm sieve is sorted entirely, and the material on 500 µm sieve is sub-sampled. Material is evenly distributed on sieve and 1/2 is removed and set aside, while the remaining 1/2 is distributed evenly on the sieve and divided in two.

- In two studies, a change in abundance and species richness was observed (Corner Brook Pulp and Paper and Abitibi-Consolidated Stephenville); however, the differences were not statistically significant.

67% (12 of 18) of studies found statistical effects in at least one of the benthic indices, and 44% (8 of 18) of mills had statistical difference in all three indices. The cause of the observed effects could not be attributed solely to currently released pulp mill effluent in many cases as other effluent discharges, different habitat, historical deposit of wood wastes/contamination, and salinity and/or tidal action confounded the interpretation.

The Cycle 1 and Cycle 2 results for the ICS are presented in Table 12. As the location of discharge outfalls, sampling locations and sampling devices changed between the cycles for some mills, the comparison between the two cycles was difficult; however, CKF/Minas Basin was the only mill with 2 cycles of no significant effects.

The conclusion from Cycle 2 benthic surveys as to whether there were "Effects" or "No Significant Effects" should be considered in conjunction with the effluent concentration at the sampling stations. As the measurement of effluent concentration at the benthic sampling sites were not available, the distance of the sampling station from outfall was used to add spatial perspective. Greater impacts (decreased abundance and number of taxa, or increased abundance of few species and decreased number of taxa) were observed at mills with stations closer to the outfall than the mills with stations further away. For the mills where the sampling stations were further away from the outfall, nutrient enrichment effects were observed (increased taxa and increased abundance). The distance of the sampling areas from the discharge outfall varied from mill to mill and ranged from within 100 m of the outfall to several kilometres (Table 11).

4.2.5 Problems/Issues in Cycle 2

The factors that confounded the interpretation of the effects of present effluent discharge on benthic communities in Cycle 2 were:

- natural variability in abundance and richness (reference area)
- difficulty in finding appropriate reference sites
- multiple discharges
- historical deposits of wood wastes

Some of these problems/issues were also identified in Cycle 1. Many studies in Cycle 2 used only one reference area and thus natural variation in benthic invertebrate communities was unknown. The observed difference between the exposure and reference areas may be due to natural variation. It was recommended by the Benthic Invertebrate Expert Working Group (BIEWG) that multiple reference areas be used in Cycle 2 (Environment Canada, 1997); however, few mills followed this recommendation. Alternative approaches to the traditional benthic studies were not available for Cycle 2 and as such, most of the problems/issues identified in Cycle 1 remained.

Table 11. Summary of the Cycle 2 Benthic Invertebrate Community Studies in the Atlantic Region (α=0.05)

Mills	Indices	No Statistical Difference	Statistical Difference ($p<0.05$)	Comments	Cycle 2 Conclusion	Location of the sampling station with respect to the Distance from Outfall
Freshwater						
Abitibi-Consolidated Grand Falls	Abundance: Richness: Diversity:	 X	X X	Total abundance (ES=8.8SD), and # of taxa (ES=1.8SD) were higher in the exposure area: moderate nutrient enrichment.	Effect - Mill Related	Exposure Area: approx. 2.5 km from N. Sewer
Fraser	Abundance: Richness: Diversity: EPT:	X X 	 X(FF) X	**Surber Study -Compared with R2** -. Statistical difference in total abundance and EPT index in NF and FF. Statistical difference in Shannon D. index in NF, and statistical difference in richness in the FF. (not a good reference site as the site receives sewage from Edmundston) **Compared with R1** – No statistical difference in abundance and diversity. EPT low in NF and FF, and richness low in FF. **Artificial Substrate Study**- no effect.	Effect	NF: 0.5 - 2.5 km FF: 11-15 km
St. Anne-Nackawic	Abundance: Richness: Diversity:	 X	X X	Increased abundance in NF Shallow area (>2SD); decrease abundance at FF Deep area (<2SD). Richness higher in NF of Deep area (<2SD).	Effect - Mill Related	NF: within 0.5 km FF: 2 - 2.7 km
Estuarine/ Marine						
Abitibi-Consolidated Stephenville	Abundance: Richness: Diversity:	X X X		No statistically significant effect observed, **but** increase in abundance and decrease in total number of taxa in NF, and increase in abundance and taxa in the North FF area observed.	No Significant Effect	24-1065 m
AV Cell	Abundance: Richness: Diversity:	X X	 X 	The correlation coefficient for pooled richness for downstream sites and distance: r^2=0.79.	Gradient Study - Effect – Mill Related C/I - reference area questionable.	U/S Gradient: 50-800 m D/S Gradient: 50-2400 m

Table 11. Summary of the Cycle 2 EEM Benthic IC Studies in the Atlantic Region (α=0.05) (continued)

Mills	Indices	No Statistical Difference	Statistical Difference ($p<0.05$)	Comments	Cycle 2 Conclusion	Location of the sampling station with respect to the Distance from Outfall
Bowater Maritimes	Abundance: Richness: Diversity:	X X X		No statistical difference in total abundance, richness and diverisy indices; **however,** significant difference in polychaete abundance observed for NF when compared to R2.	No Significant Effect	NF: within 300 m FF: not sampled
Bowater Mersey	Abundance: Richness: Diversity:		X X X	High densities in NF, FF and FFF. Low mean number of taxa in NF and FF. Despite the impairment in NF, the conditions have improved since Cycle 1.	Effect - Mill Related: confounded by historical deposit of woodwaste (NF, FF)	NF: approx. 200 m FF: approx. 750 m FFF: approx. 1500 m
CKF/Minas Basin	Abundance: Richness: Diversity:	X X X		No statistical difference was observed when the number of taxa for Transect 2 was regressed according to distance. A reduction in abundance and number of taxa with increasing distance from outfall was observed; however, TOC, sulfide and redox measurements did not support nutrient enrichment effect.	No Significant Effect	0-200 m (north and south)
Corner Brook Pulp and Paper	Abundance: Richness: Diversity:	X X X		No statistically significant effects **but** decreased abundance and mean number of taxa were observed in the NF and FF. FFW dominated by Capitella.	No Significant Effect	NF: approx. 50 m FF: approx. 200 m FFW: approx. 200 m
Irving Paper	Abundance: Richness: Diversity:		X X X	Low abundance, number of species, diversity and biomass in NF. NF in southwest direction devoid of organisms.	Effect - Mill related	NF: approx. 150 m Stations from 0 to 1.5 km
Irving Pulp & Paper	Abundance: Richness: Diversity:	X X X		No statistical effect when indices were regressed over distance from outfall; **however,** diversity and evenness decreased with increasing distance from mill effluent outfall: mild nutrient enrichment.	No Significant Effect	0-5.6 km

Table 11. Summary of the Cycle 2 EEM Benthic IC Studies in the Atlantic Region (α=0.05) (continued)

Mills	Indices	No Statistical Difference	Statistical Difference (p<0.05)	Comments	Cycle 2 Conclusion	Location of the sampling station with respect to the Distance from Outfall
Kimberly-Clark NS	Abundance: Richness: Diversity:	X X X		No statistical difference in total abundance, richness and diversity; **however,** high polychaete density in FF (p=0.015). Nutrient enrichment effect in intertidal area.	No Significant Effect	NF: approx. 500 m FF: approx. 1000 m
L.P. East River	Abundance: Richness: Diversity:		X X X	Decrease in abundance, richness and diversity in the NF. 56% reduction in biomass in NF compared to Reference. Extent of fibre mat <150 m in southwest direction.	Effect - Mill Related: confounded by historical deposit of woodwaste.	NF: 37 - 96 m FF: 129 m
Lake Utopia	**Impoundment:** Abundance: Richness: Diversity: **Estuary:** Abundance: Richness: Diversity:	**See comments**	 X X X	**Impoundment**: All samples had low abundance, number of species and diversity. There was no relationship with benthic indices and the distance from the outfall. All the stations in the impoundment were affected. The impoundment has layers of historical deposit of woodwaste. **Estuary**: Abundance, number of taxa and species diversity increased with increasing distance from the outfall.	Effect - Mill Related: confounded by historical deposits of wood waste in the Impoundment, and Salinity/tidal effect for the estuary.	Imp: 0-3.25 km Est: 3.5-5.45 km
Smurfit Stone Container	Abundance: Richness: Diversity:		X X X	Decreased abundance, richness and diversity in NF (ES >2SD). NF comprised of pollution tolerant taxa.	Effect - Mill Related	C/I: up to 400 m Gradient: <100 to 4 km
Stora Enso	Abundance: Richness: Diversity:		X X X	Abundance in FF statistically different from RB. Number of Taxa in all the exposure areas is statistically different from RB. Diversity in NF statistically different from RA.	Effect - Mill Related: confounded by historical deposits of woodwaste	NF: <100 m FF: approx. 500 m

Table 11. Summary of the Cycle 2 EEM Benthic IC Studies in the Atlantic Region (α=0.05) (continued)

Mills	Indices	No Statistical Difference	Statistical Difference (p<0.05)	Comments	Cycle 2 Conclusion	Location of the sampling station with respect to the Distance from Outfall
UPM-Kymmene Groundwood	Abundance: Richness: Diversity:		X X X	Low abundance, richness and diversity at far-field (station G6) and far-far-field (station G4).	Effect - Unknown cause: confounded by salinity/tidal effect	NF: approx. 125-225 m FF: approx. 550-575 m
UPM-Kymmene Kraft	Abundance: Richness: Diversity:		X X X	Decreased abundance, species richness and diversity in the NF in deep water.	Effect- Unknown cause: confounded by salinity/tidal effect	NF-shallow: approx.185-440 m NF-deep: approx. 160-825 m

Table 12. Results of Cycle 1 and Cycle 2 ICS in the Atlantic Region

Mills	Cycle 1 Conclusion	Cycle 2 Comments (statistical significant difference at p<0.05)	Cycle 2 Conclusion
Freshwater			
Abitibi-Consolidated Grand Falls	Productivity in terms of species and organisms similar b/w exposure and reference.	Total abundance (ES=8.8SD), and # of taxa (ES=1.8SD) were higher in the exposure area: moderate nutrient enrichment.	Effect - Mill Related
Fraser	Near field dominated by Naidid worms and had high species richness.	**Surber Study: Compared with R2** (immediately upstream of mill outfall) - Statistical difference in total abundance and EPT index for NF and FF. Significant difference in Shannon W. Index for NF and significant difference in richness in FF (not a good reference site as the site receives sewage from Edmunston). **Compared with R1** – No statistical difference in abundance and diversity. EPT low in NF and FF, and richness low in FF. **Artificial Substrate Study**: No effect.	Effect
St. Anne-Nackawic	NF: increase in abundance of pollution tolerant species. Mayfly (pollution sensitive) abundant in reference and absent in exposure	Increased abundance in NF Shallow area (>2SD); decrease abundance at FF Deep area (<2SD). Richness higher in NF of Deep area (<2SD).	Effect - Mill Related
Estuarine/Marine			
Abitibi-Consolidated Stephenville	Amphipods & Oligochaetes (pollution sensitive) present	No statistically significant effect observed, **but** increase in abundance and decrease in total number of taxa in NF, and increase in abundance and taxa in the North FF area observed.	No Significant Effect
AV Cell	N/A	The correlation coefficient for the pooled richness and distance for the south transect is significant (r^2=0.8).. The reference area for the C/I study design is questionable and as such analysis was not conducted.	Effect: Mill Related
Bowater Maritimes	Low richness and increased abundance in Near Field. Dominated by pollution tolerant species.	No statistical difference in total abundance, richness and diversity indices; **however,** significant difference in polychaete abundance observed for NF when compared to R2.	No Significant Effect

Table 12. Results of Cycle 1 and Cycle 2 ICS in the Atlantic Region (continued)

Mills	Cycle 1 Conclusion	Cycle 2 Comments	Cycle 2 Conclusion
Bowater Mersey	NF almost devoid of invertebrates and only species present were Oligochaetes (pollution tolerant)	High densities in NF, FF and FFF. Low mean number of taxa in NF and FF. Despite the impairment in NF, the conditions have improved since Cycle 1.	Effect - Mill Related: confounded by historical deposit of woodwaste (NF and FF).
CKF/Minas Basin	No differences in community with respect to effluents from mill	No statistical difference was observed (p=0.08, power=0.412, alpha= 0.05) for number of taxa for Transect 2 when it was regressed according to distance. Observed reduction in abundance and number of taxa with increasing distance from outfall; however, TOC, sulfide and redox measurements did not support nutrient enrichment effect.	No Significant Effect
Corner Brook Pulp and Paper	Low abundance and richness in exposure area. Dominance of pollution tolerant species.	No statistically significant effects **but** decreased abundance and mean number of taxa are observed in the NF and FF. FFW dominated by Capitella.	No Significant Effect
Irving Paper	Low # species and organisms	Low abundance, number of species, diversity and biomass in NF. NF in southwest direction devoid of organisms.	Effect – Mill related
Irving Pulp & Paper	High abundance in exposure area.	No statistical effect when indices were regressed over distance from outfall; **however,** diversity and evenness decreased with increasing distance from mill effluent outfall: mild nutrient enrichment.	No Significant Effect
Kimberly-Clark NS	Low abundance.	No statistical difference in total abundance, richness and diversity; **however,** high polychaete density in FF (p=0.015). Nutrient enrichment effect in Intertidal area.	No Significant Effect
L.P. East River	Reduced abundance and richness in Near Field; Reduced richness and increased abundance in Far Field.	Decrease in abundance, richness and diversity in the NF. Extent of impact <200 m.	Effect – Mill Related: confounded by historical deposit of woodwaste

Table 12. Results of Cycle 1 and Cycle 2 ICS in the Atlantic Region (continued)

Mills	Cycle 1 Conclusion	Cycle 2 Comments	Cycle 2 Conclusion
Lake Utopia	NF had reduced richness and abundance, dominated by pollution tolerant species. FF3 low richness, dominated by Polychaetes.	**Impoundment**: All samples had low abundance, number of species and diversity. There was no relationship with benthic indices and the distance from the outfall. All the stations in the impoundment were affected. The impoundment has layers of historical deposit of woodwaste. **Estuary**: Abundance, number of taxa, and species diversity incresed with increasing distance from the outfall.	Effect – Mill Related: confounded by historical deposits of wood waste (Imp.)
Smurfit Stone Container	Low richness and abundance in Near Field. Dominated by pollution tolerant species.	Decreased abundance, richness and diversity in NF (ES >2SD). NF comprised of pollution tolerant taxa.	Effect – Mill Related: reference area questionable
Stora Enso	Richness and abundance extremely low.	Abundance in FF statistically different from RB. Number of taxa in all the exposure areas is statistically different from RB. Diversity in NF statistically different from RA.	Effect – Mill Related: counfounded by historical deposits of woodwaste
UPM-Kymmene Groundwood	Low # species and organisms	Low abundance, richness and diversity at far-field (station G6) and far-far-field (station G4).	Effect – Unknown cause: confounded by salinity
UPM-Kymmene Kraft	Little difference b/w reference and exposure areas	Decreased abundance, species richness and diversity in the NF in deep water.	Effect- Unknown cause: confounded by salinity

5.0 Effluent Sublethal Toxicity Tests

Another component of Pulp and Paper EEM is the assessment of sublethal toxicity of effluent through the use of effluent toxicity tests, which involve exposure of organisms to a series of effluent dilutions. In Cycle 2, mills were required to conduct semi-annual sublethal toxicity tests on their process effluent: once during the summer test period (July-October) and once during the winter period (January-April). Organisms from three different trophic levels (fish, invertebrate, and aquatic plant) were used to assess the potential toxicity of effluent. The recommended methodologies are presented in Table 13.

Table 13. EC Recommended Sublethal Toxicity Tests and Methodologies

Test Description	Receiving Environment	Test Species	Acceptable Methods
Fish early life stage development test	Marine	*Menidia beryllina* (inland silverside) or *Atherinops affinis* (topsmelt)	U.S. EPA 1994 and 1995
	Freshwater	*Pimephales promelas* (fathead minnow)	EC 1992a or EC 1997c
Invertebrate Reproduction test	Marine	Echinoids (sea urchins or sand dollars	EC 1992b
	Freshwater	*Ceriodaphnia dubia*	EC 1992c
Plant toxicity test	Marine	*Champia parvula*	U.S. EPA 1994
	Freshwater	*Selenastrum capricornutum*	EC 1992d

In Cycle 2, all mills discharging into freshwater environment used *Pimephales promelas, Ceriodaphnia dubia,* and *Selenastrum capricornutum.* The mills that discharge into an estuarine or marine environment used *Menidia beryllina, Lytechinus pictus,* and *Champia parvula* as their test organisms.

5.1 Objectives

The objectives of sublethal toxicity testing in Cycle 2 were (Environment Canada, 1998):

1. to contribute to the field program as part of the weight of evidence approach
2. to compare process effluent quality between mill types and measure changes in effluent quality as a result of effluent treatment and process changes, and
3. to contribute to the understanding of the relative contributions of the mill in multiple discharge situations (i.e., municipal waste-water, other industrial discharges, historical contamination).

5.2 Results and Discussion

Most mills conducted six sets of sublethal toxicity tests in Cycle 2 and the data are presented in Appendix 2. Some exceptions were:

- The joint study conducted by CKF/Minas Basin used a composite effluent sample according to discharge volumes from the 2 mills for the sublethal toxicity tests to assess the potential toxicity of their combined effluent as the mills discharge effluent in close proximity to each other.

- Abitibi-Consolidated Grand Falls conducted the sublethal toxicity tests on three discharges: ASB, North Sewer and #3 Sewer to keep track of their effluent quality with respect to different waste streams.

- St. Anne-Nackawic conducted *Daphnia* tests on their intake water to ensure that the observed toxicity in their effluent is in fact due to the activities within the mill.

- Due to a labour dispute, the Abitibi-Consolidated mills in Newfoundland were shut down for a period in 1998 (June-November) and only conducted 5 sets of sublethal toxicity tests.

In general, the Cycle 2 sublethal toxicity test results indicated the following:

- an improvement in effluent quality from Cycle 1 to Cycle 2, as can be seen in Figure 3 by the increase in IC_{25} concentrations in all the organisms tested.

- most of the final effluent discharging into estuarine/marine systems did not elicit a toxic response in fish (11 of 15 mills); however, toxic responses are still seen in the algae and invertebrates (all mills).

- for mills discharging effluent into a freshwater system, some toxicity is still seen in all the organisms tested.

Results of the tests were analyzed in terms of IC_{25} (concentration resulting in a 25% inhibition) values. A comparison of Cycle 1 and Cycle 2 IC_{25} data for all organisms tested indicated a statistically significant difference in IC_{25} concentrations ($p<0.0001$). Even though great improvements in effluent quality are seen in Cycle 2, the effluent from most of the mills in the Atlantic Region still elicits toxic responses in invertebrates and algae.

The reduced toxicity as seen in the increased IC_{25} values in Cycle 2 from Cycle 1 is probably related to the installation of secondary treatment at 9 mills and the improvement/upgrading of existing treatment systems at most of the remaining mills. By Cycle 2, almost all the mills in the Atlantic Region had installed a secondary treatment system except two: Irving Pulp and Paper, and CKF/Minas Basin. However, the Irving Pulp and Paper mill has installed an Oxygen Delignification System and a Reverse Osmosis System to improve effluent quality. The CKF/Minas Basin achieves compliance with the PPER using only primary treatment.

There is no prescribed 'toxic criteria' to describe the results of the toxicity tests, as effluent toxicity on aquatic organisms depends on the site specific receiving environment. In Cycle 1, a qualitative scale with 4 categories was used (Parker and Smith, 1999). For Cycle 2, a similar qualitative scale is used with the addition of the <1% category to highlight the most toxic effluent and the most ecologically relevant concentration in the receiving environment (Table 14).

Figure 3. Sublethal Toxicity of Pulp Mill Effluent: Cycle 1 vs Cycle 2 (mean IC_{25} and SD)

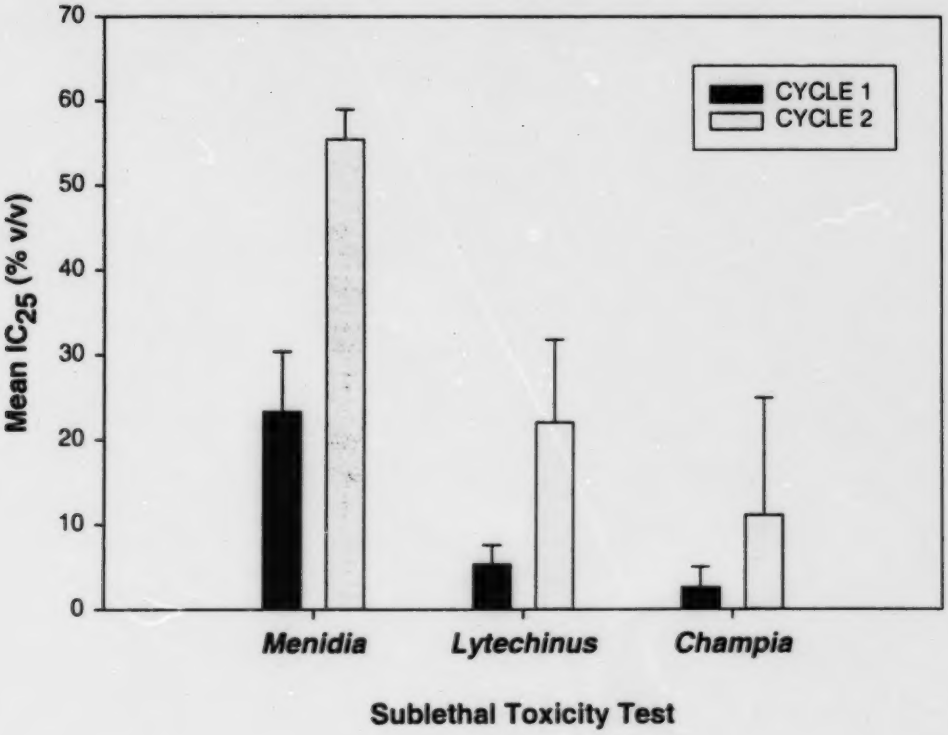


Figure 4. Effluent Toxicity Levels for the mills that discharge into a Freshwater Environment

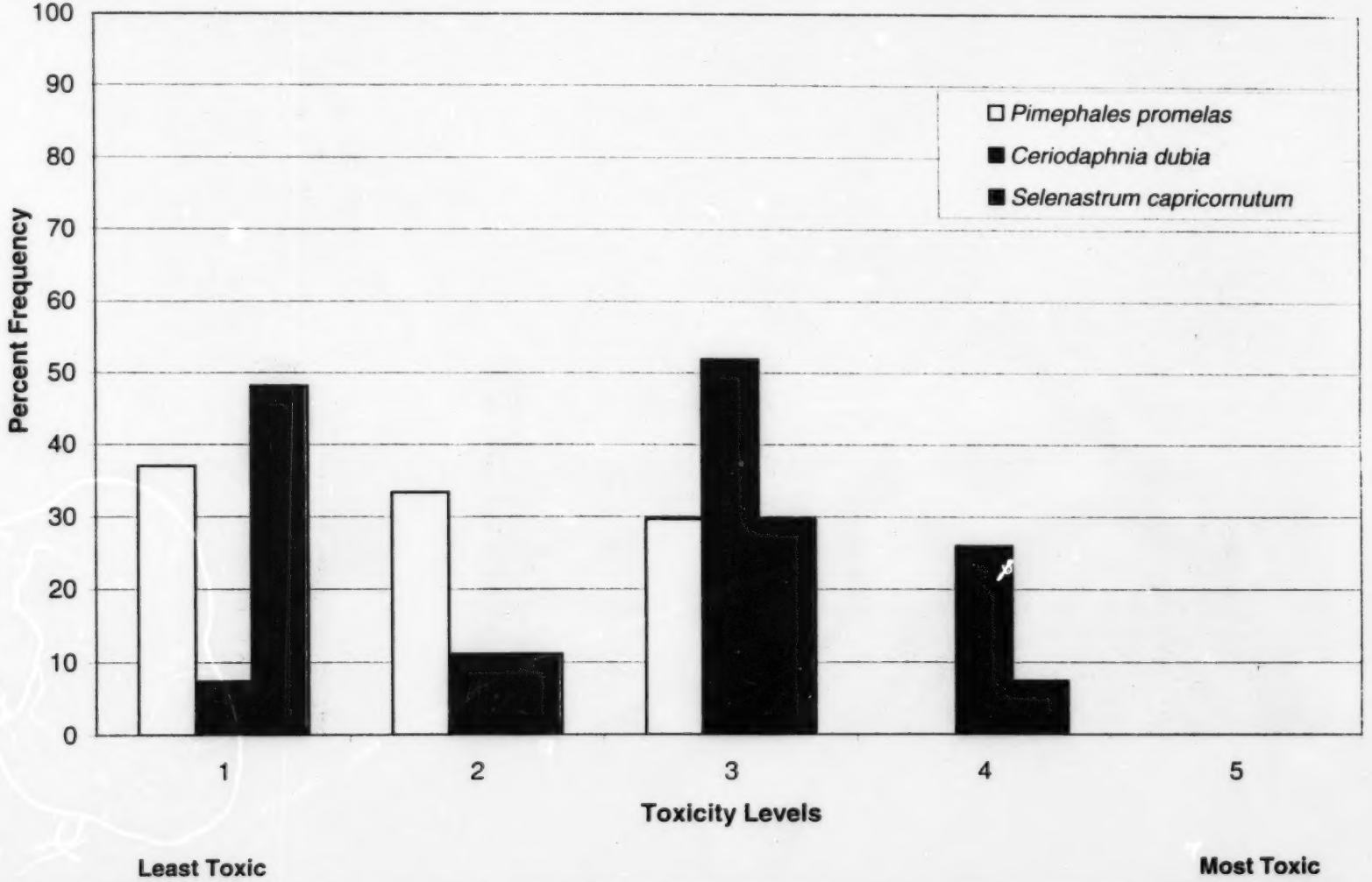


Figure 5. Effluent Toxicity Levels for the mills that discharge into an Estuarine/Marine Environment

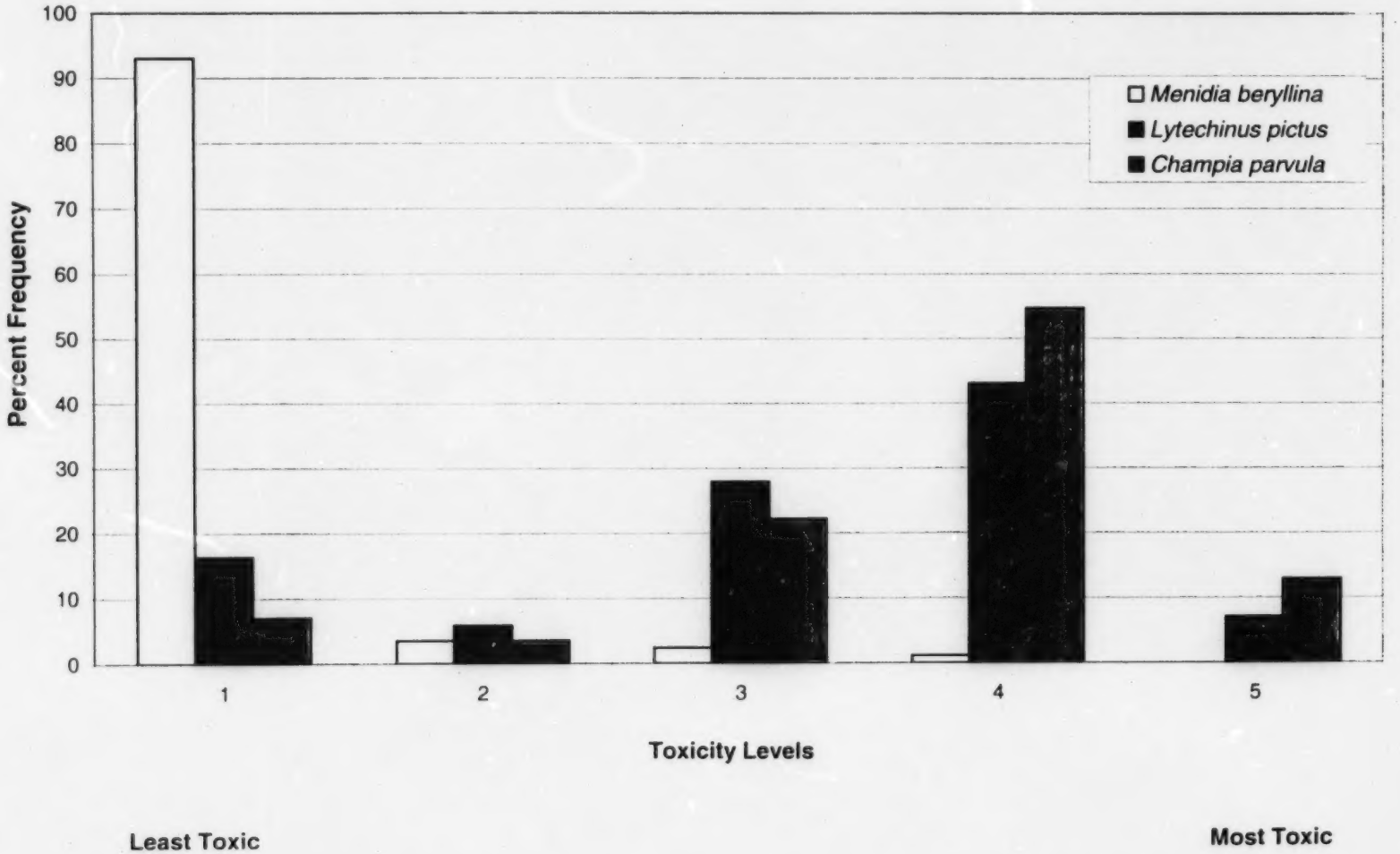


Figure 6. Sublethal Toxicity of *Lytechinus pictus* by Mill Process Type

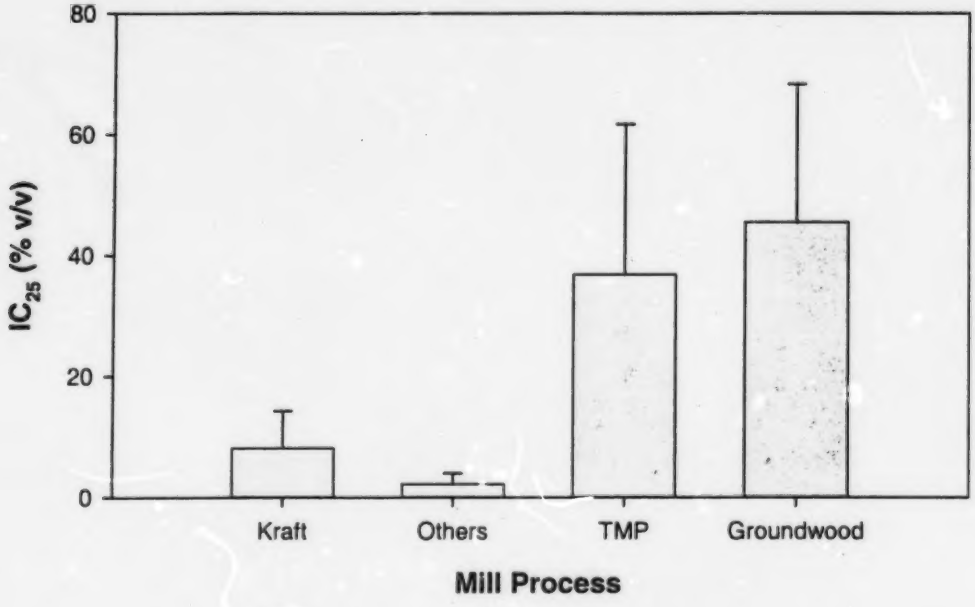


Figure 7. Sublethal Toxicity of *Champia parvula* by Mill Process Type

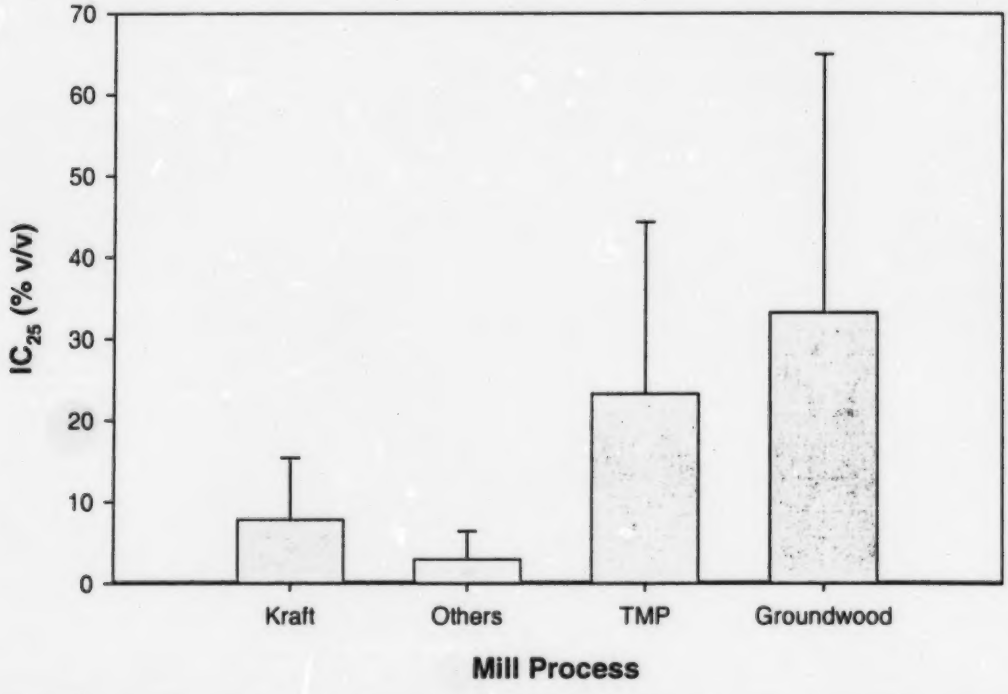


Table 14. Description of Sublethal Toxicity Levels and how they relate to GM-IC_{25}.

Toxicity Level	Description	Freshwater Test (% Effluent)	Estuarine/Marine Test (% Effluent)
1	No Response	≥100	≥67
2	Low Toxicity	≥50 and <100	≥50 and <67
3	Moderate Toxicity	≥15 and <50	≥15 and <50
4	High Toxicity	≥1 and <15	≥1 and <15
5	Severe Toxicity	<1	<1

Table 15. Cycle 2 Pulp Mill Effluent Toxicity (IC_{25}) Characterization

Test	Number of Tests	100%	≥50 and <100%	≥15 and <50%	≥1 and <15%	<1%	No Record
Freshwater:							
Pimephales promelas	27	10	9	8	0	0	0
Ceriodaphnia dubia	27	2	3	14	7	0	1
Selanastrum capricornutum	27	13	3	8	2	0	1
Test:	**Number of Tests**	**>67%**	**≥50 and <67%**	**≥15 and <50%**	**≥1 and <15%**	**<1%**	**No Record**
Estuarine/Marine							
Menidia beryllina	86	80	3	2	1	0	0
Lytechinus pictus	86	14	5	24	37	6	0
Champia parvula	86	6	3	19	47	11	0

Note: concentrated brine is added to marine effluent tests for salinity adjustment, hence the maximum effluent concentration is >67% for marine tests.

Freshwater

In freshwater sublethal toxicity tests, the pulp mill effluent elicited the greatest toxic response in the *C. dubia*, followed by *S. capricornutum*, and no response in the *P. promelas*.

26% (7of 27) of the *C. dubia* tests conducted had results in the high sublethal toxicity category (Toxic Level 4); 7% (2 of 27) of *S. capricornutum* results fell in this range, and none of the fish results. It should also be noted that none of the effluent tested was severely toxic (Toxic Level 5) to any of the organisms tested, which is an improvement from Cycle 1 (Table 15 and Figure 4). In Cycle 1, 10% (4 of 44) of effluent tests conducted were severely toxic, and 20% (8 of 44) were highly toxic (Toxic Level 4).

The geometric mean (GM) IC_{25} in Cycle 2 ranged from 15.9% to 39% for the *C. dubia* test; 42.8% to 100% for the *P. promelas* test; and 34.6% to 71.4% for the *S. capricornutum* test (Table 17). Standard deviation ranged from 11.7% to 47.4% for the *C. dubia* test; 8.86% to 32.2% for the *P. promelas* test; and 27.5% to 47.9% for the *S. capricornutum* test (Appendix 2). A large variability is observed between the samples in the freshwater tests. The cause of the variability may include inconsistent effluent

quality and laboratory variability as different laboratories were used in Cycle 2 for the same test by some mills.

The maximum zone of potential sublethal effects (the extent of 25% inhibition in the sublethal endpoint being measured in the environment, calculated by using the geometric mean IC_{25}, and the zone of 1% effluent dilution distance) for freshwater environment ranged from 25 to approximately 300 metres in Cycle 2. The actual zone of impact in the receiving environment is not clear as sublethal toxicity tests are not designed to address the cumulative and chronic effects of effluent on organisms. The maximum exposure period for the sublethal toxicity tests is 7 days.

A more detailed data analysis on the toxicity test results for mills that discharge into a freshwater receiving environment was not conducted due to the small sample size (n=3).

Estuarine/Marine

In estuarine/marine tests, pulp mill effluent elicited the greatest toxic response in the *C. parvula*, the marine algae, and the least response in the *M. beryllina*, the marine fish.

The Cycle 2 results indicated that 93% of tests (80 of 86) conducted on *M. beryllina* gave no toxic responses (Toxic Level 1, IC_{25}>67%), while 7% of tests (6 of 86) conducted on *L. pictus*, and 13% of tests (11 of 86) conducted on *C. parvula* were severely toxic (Toxic Level 5 = <1% effluent concentration [Figure 5]). Pulp mill effluent from 11 of 15 mills that discharge into estuarine/marine systems in the Atlantic Region did not elicit a toxic response in 7-day fish survival and growth test.

The cause of observed toxicity of pulp mill effluent to invertebrates and algae is unclear, but the test endpoint measured for these species is reproduction while the endpoint for fish is survival and growth. The data indicate that the pulpmill effluent affects reproduction in invertebrates and algae. As current sublethal toxicity component of EEM does not measure fish reproduction, the effect of pulpmill effluent on fish reproduction is unknown.

The sublethal toxicity data on *L. pictus* and *C. parvula* were examined to assess the effects of seasons. The data were differentiated into different mill processes and effluent treatment types. A seasonal difference was detected for ASB effluent treatment type for *C. parvula* sublethal toxicity data (Table 16) with effluent eliciting a greater sublethal toxicity during summer. The cause of this toxicity is not clear. This difference disappeared when the seasonal effect was combined with the mill factor. As the number of samples for this analysis was limited, an analysis using the national data set is recommended. A seasonal difference was not found for *L. pictus* for ASB effluent treatment type or in different mill process types.

The geometric mean IC_{25} and 1% zone of potential sublethal effects are presented in Tables 19-21. The geometric mean IC_{25} ranged from 35.87% to >67% for *M. beryllina*, 0.89% to 61.66% for *L. pictus* and 0.91% to 46.06% for *C. parvula*.

The data indicate a large variability between samples (Appendix 2). The cause of the variability may include variable effluent quality including seasonal variation in effluent treatment and laboratory variability, as different technicians and laboratories were used by some mills in Cycle 2.

The national database should be used to assess the seasonal variability in *C. parvula* with ASB treatment system. Other factors that lead to the variability of sublethal toxicity results in Cycle 2 are not known, but are probably related to changes in mill process and effluent treatment and laboratory variablity.

5.2.1 Mill Process and Effluent Treatment

The degree of sublethal toxicity of effluent to invertebrates and algae may be related to the type of mill process, bleaching process (if any), mill furnish, mill product and the secondary effluent treatment process used. A detailed multi-variate analysis would be required to determine the key factors influencing sublethal toxicity of pulp mill effluent using the national data set. However, the regional sublethal toxicity data were examined to see if different types of mill process influence sublethal toxicity of pulp mill effluent. Due to the small sample size, the results of this analysis should be used with caution.

A statistical analysis was not conducted on the effect of effluent treatment on sublethal toxicity as the sample size was limited (numerous treatment processes are used in the region (Table 3), and few mills used the same effluent treatment process).

5.2.2 Mill Processes and Sublethal Toxicity of Pulp Mill Effluent

Various mill processes used by the pulp and paper mills in the Atlantic Region were differentiated into 4 classes for comparison: Kraft, TMP, Groundwood and Others (Chemi-Thermo Mechanical Pulp, Dissolving Grade Sulfite and Neutral Sulphite Semi-chemical). The classes of mill processes were examined with the marine invertebrate and algae sublethal toxicity data to assess how different mill processes affect sublethal toxicity. Figures 6 and 7 indicate that groundwood and TMP process effluent have lower sublethal toxicity than the Kraft and Other process effluents for both *L. pictus* and *C. parvula* with Other process effluent being the most toxic.

5.2.3 1% Effluent Zone and the Zone of Potential Sublethal Effects

The 1% effluent zone and the zone of potential sublethal effects (extent of1% effluent/geometric mean IC_{25}) as reported in the Cycle 2 reports are provided in Tables 18 through 21. The 1% effluent plume zone ranged from <250 metres to 5 300 metres depending on site-specific conditions. The data (Tables 19-21) indicated that the maximum zone of potential sublethal effects (MZPSE) was quite localized for most mills with:

- ten mills having the MZPSE less than100 metres,
- four mills having MZPSE greater than 100 and less than or equal to 1 000 metres,
- two mills having MZPSE greater than 1 000 metres, and
- for two mills, MZPSE was not calculated due to poor plume delineation.

As currents, wind, and site specific factors control the 1% dilution zone, the use of 1% effluent plume zone to delineate the zone of potential impact should be used with caution. The MZPSE is only as good as the plume study. In spite of this limitation, the MZPSE indicated that potentially impacted areas are generally confined to the vicinity of the outfalls. The sublethal toxicity tests are not designed to address chronic and

cumulative effects of effluent in the receiving environment, and thus may not accurately reflect the effects observed in the field. A study should be undertaken to assess the relationship between the sublethal toxicity data and the field effects.

Table 16. Seasonal Difference in Sublethal Toxicity for ASB Effluent Treatment System

Champia parvula

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
MILL	2298.547	4	574.637	4.651	0.009
SEASON	1398.6	1	1398.6	11.32	0.003
MILL*SEASON	1156.177	4	289.044	2.339	0.092
Error	2347.501	19	123.553		

5.2.4 Laboratories and Sublethal Toxicity Reports

The mills in the Atlantic Region used the following laboratories to conduct the sublethal toxicity tests for EEM:

- Beak International Inc., Ontario
- ESG, Ontario
- Bodycote Technitrol Inc., Quebec
- SRC, Saskatchewan

All the tests were conducted in a satisfactory manner and were accepted by Environment Canada except one. All the mills in the Atlantic Region, except two mills on 2 occasions, submitted the sublethal toxicity test results within 90 days of test completion as required under the PPER.

Table 17. **Comparison of Cycle 1 and Cycle 2 sublethal toxicity geometric mean (GM) IC_{25} for mills that discharge into a freshwater**

Mills	Cycle 1						Cycle 2					
	GM IC_{25}	Lowest IC_{25}	Highest IC_{25}	Toxicity Level	Max. Extent of 1% Plume (m)	Potential Zone of Effect (m)	GM IC_{25}	Lowest IC_{25}	Highest IC_{25}	Toxicity Level	Max. Extent of 1% Plume (m)	Potential Zone of Effect (m)
Ceriodaphnia dubia (reproduction)												
Abitibi-Consolidated Grand Falls (#3 Sewer)	N/a	n/a	n/a	n/a	n/a	n/a	15.94	8	54.1	3	n/a	n/a
Abitibi-Consolidated Grand Falls (ASB)	N/a	n/a	n/a	n/a	n/a	n/a	39.04	26.9	49.8	3	n/a	n/a
Abitibi-Consolidated Grand Falls (North Sewer)	N/a	n/a	n/a	n/a	n/a	n/a	29.85	11	100	3	n/a	n/a
Fraser Papers	7.26	2.6	17.6	4	5000	689	16.75	1.9	33.6	3	5000	299
St. Anne-Nackawic	13.41	9.2	18.1	4	2500	186	29.26	13.1	60.7	3	2500	85
Pimephales promelas (growth)												
Abitibi-Consolidated Grand Falls (#3 Sewer)	N/a	n/a	n/a	n/a	n/a	n/a	60.4	33.8	80.2	2	n/a	n/a
Abitibi-Consolidated Grand Falls (ASB)	N/a	n/a	n/a	n/a	n/a	n/a	64.9	25	100	2	n/a	n/a
Abitibi-Consolidated Grand Falls (North Sewer)	N/a	n/a	n/a	n/a	n/a	n/a	73.2	39.6	100	2	n/a	n/a
Fraser Papers	21.48	18	26.1	3	5000	233	42.8	33.8	59.6	3	5000	117
St. Anne-Nackawic	91.90	71.4	>100	2	2500	27	100	100	100	1	2500	25
Selenastrum apricornutum (growth)												
Abitibi-Consolidated Grand Falls (#3 Sewer)	N/a	n/a	n/a	n/a	n/a	n/a	50.4	16.6	100	2	n/a	n/a
Abitibi-Consolidated Grand Falls (ASB)	N/a	n/a	n/a	n/a	n/a	n/a	34.6	3.9	100	3	n/a	n/a
Abitibi-Consolidated Grand Falls (North Sewer)	N/a	n/a	n/a	n/a	n/a	n/a	71.4	18.6	100	2	n/a	n/a
Fraser	9.00	<1.0	24.5	4	5000	556	65.1	41	100	2	5000	77
St. Anne-Nackawic	N/a	n/a	n/a	n/a	2500	n/a	38.7	12.9	100	3	2500	65

Table 18. Comparison of Cycle 1 and Cycle 2 *Menidia beryllina* sublethal toxicity geometric mean (GM) IC_{25} for mills that discharge into an estuarine/marine environment

Mills	Cycle 1						Cycle 2					
	GM IC_{25}	Lowest IC_{25}	Highest IC_{25}	Toxicity Level	Max. Extent of 1% Plume (m)	Potential Zone of Effect (m)	GM IC_{25}	Lowest IC_{25}	Highest IC_{25}	Toxicity Level	Max. Extent of 1% Plume (m)	Potential Zone of Effect (m)
Abitibi-Consolidated Stephenville	>67	>67	>67	1	600*	9	>67	>67	>72	1	600*	9
AV Cell	N/a	n/a	N/a	n/a	3300	n/a	63.31	60.3	>67	2	3300	49
Bowater Maritimes	4.42	3.8	4.8	4	350	79	>67	>67	>72	1	350	5
Bowater Mersey	4.04	2	7	4	1000	248	>67	>67	>72	1	1000	15
CKF/Minas Basin	>67	>67	>67	1	<250	4	>67	>67	>71	1	<250	4
Corner Brook	11.46	7.3	22	4	425	37	>67	>67	>71	1	425	6
Irving P&P	10.97	5.4	19.07	4	5300	483	35.87	2.2	>67	3	5300	79
Irving Paper	6.23	3.6	10.67	4	n/a	n/a	>67	>67	>71	1	n/a	n/a
Kimberly-Clark	64.88	58.9	>67	2	2000	31	>67	>67	>72	1	2000	30
L.P. East River	56.83	34.67	>67	2	200	4	>67	>67	>71	1	200	3
Lake Utopia	n/a	n/a	N/a	n/a	4000	60	55.54	19.8	>71	2	4000	60
Smurfit Stone Container	26.87	7	>67	3	2000	74	>67	>67	>73	1	2000	30
Stora Enso	3.60	2.2	7.3	4	450	125	>67	>67	>71	1	450	7
UPM-Kymmene Groundwood	8.84	3.32	43.67	4	250	28	>67	>67	>73	1	250	4
UPM-Kymmene Kraft	58.11	50.12	>67	2	325	4	66	60.5	>72	2	325	5

* based on visible effluent plume observed on July 14 and 16, 1999.

Table 19. **Comparison of Cycle 1 and Cycle 2 *Lytechinus pictus* sublethal toxicity geometric mean (GM) IC_{25} for mills that discharge into an estuarine/marine environment**

	Cycle 1						Cycle 2					
Mills	GM IC_{25}	Lowest IC_{25}	Highest IC_{25}	Toxicity Level	Max. Extent of 1% Plume (m)	Potential Zone of Effect (m)	GM IC_{25}	Lowest IC_{25}	Highest IC_{25}	Toxicity Level	Max. Extent of 1% Plume (m)	Potential Zone of Effect (m)
Abitibi-Consolidated Stephenville	2.09	0.73	5	4	600*	287	25.77	9.08	43.6	3	600*	23
AV Cell	n/a	n/a	n/a	n/a	3300	n/a	3.45	2.6	4.53	4	3300	957
Bowater Maritimes	1.71	0.73	2.47	4	350	205	27.81	9.77	47.1	3	350	13
Bowater Mersey	10.4	10.07	10.53	4	1000	96	35.21	7.39	>67	3	1000	28
CKF/Minas Basin	30.23	20.07	37.54	3	<250	8	61.66	42	>71	2	<250	4
Corner Brook	1.93	0.03	15.07	4	425	220	61.15	40.1	>70	2	425	7
Irving P&P	1.08	0.16	4.71	4	5300	4907	13.44	4.84	20.2	3	5300	394
Irving Paper	8.72	5.32	14.47	4	n/a	n/a	42.06	10.5	>67	3	n/a	N/a
Kimberly-Clark	2.19	0.8	5.47	4	2000	913	5.13	3.63	7.92	4	2000	390
L. P. East River	1.45	0.6	2.6	4	200	138	4.23	1.75	13.2	4	200	47
Lake Utopia	n/a	n/a	n/a	n/a	4000	n/a	0.89	0.37	4.92	5	4000	4494
Smurfit Stone Container	1.66	1.07	2.6	4	2000	1205	1.76	0.66	5.6	4	2000	1136
Stora Enso	0.2	0.09	0.47	5	450	2250	4.93	0.65	19	4	450	91
UPM-Kymmene Groundwood	6.1	5.04	7.27	4	250	41	40.01	17.2	>72	3	250	6
UPM-Kymmene Kraft	1.38	0.73	3.14	4	325	295	2.93	0.52	9.5	4	325	112

* based on visible effluent plume observed on July 14 and 16, 1999.

Table 20. Comparison of Cycle 1 and Cycle 2 *Champia parvula* sublethal toxicity geometric mean (GM) IC_{25} for mills that discharge into an estuarine/marine environment

	Cycle 1						Cycle 2					
Mills	**GM IC_{25}**	**Lowest IC_{25}**	**Highest IC_{25}**	**Toxicity Level**	**Max. Extent of 1% Plume (m)**	**Potential Zone of Effect (m)**	**GM IC_{25}**	**Lowest IC_{25}**	**Highest IC_{25}**	**Toxicity Level**	**Max. Extent of 1% Plume (m)**	**Potential Zone of Effect (m)**
Abitibi-Consolidated Stephenville	2.48	0.73	9.8	4	600*	242	7.88	1.77	33.54	4	600*	76
AV Cell	n/a	n/a	n/a	N/a	3300	n/a	5.55	3.08	13.81	4	3300	595
Bowater Maritimes	<2.9	<2.9	<2.9	4	350	121	11.51	0.24	>68.1	4	350	30
Bowater Mersey	2.28	0.67	4.73	4	1000	438	14.89	7.1	38.9	3	1000	67
CKF/Minas Basin	6.47	3.07	9.67	4	<250	39	16.33	5	31.11	3	<250	15
Corner Brook	2.64	1.47	3.53	4	425	161	46.06	23.7	>68	3	425	9
Irving P&P	1.59	0.85	4.27	4	5300	3343	8.63	1.36	33.5	4	5300	614
Irving Paper	2.16	0.76	6.89	4	n/a	n/a	17.29	9.24	63.4	3	n/a	n/a
Kimberly-Clark	1.59	3.06	12.82	4	2000	1262	5.47	2.87	13.2	4	2000	366
L.P. East River	5.35	0.67	16.67	4	200	37	6.49	0.47	29.92	4	200	31
Lake Utopia	n/a	n/a	n/a	N/a	4000	n/a	1.71	0.45	4.96	4	4000	2339
Smurfit Stone Container	0.30	0.16	0.93	5	2000	6599	0.91	0.22	5.02	5	2000	2198
Stora Enso	0.43	<0.13	7.8	5	450	1036	6.52	0.24	>70.1	4	450	69
UPM-Kymmene-Groundwood	1.85	0.84	4.33	4	250	135	14.49	0.56	>71.9	4	250	17
UPM-Kymmene-Kraft	3.51	<2.95	5.9	4	325	87	2.71	0.19	8.53	4	325	150

* based on visible effluent plume observed on July 14 and 16, 1999.

6.0 RESEARCH EFFORTS BY THE PULP AND PAPER INDUSTRY IN THE ATLANTIC REGION

Confounding factors in the receiving environment (other effluent discharges, historical deposit of wood wastes, and historical sediment contamination), and the degree of exposure of fish to effluent made the interpretation of the Cycle 1 data difficult. Several mills in the Atlantic Region were proactive in collaborating with Environment Canada to assess the effects of their effluent using alternative studies. The alternative studies included:

- mesocosm (artificial stream systems) for benthic community and fish studies
- caged bivalve studies
- fathead minnow life cycle bioassays
- caged fish studies
- artificial substrate samplers

6.1 Mesocosm Study using Mummichogs

Mesocosm studies using mummichogs took place at Irving Paper, Irving Pulp and Paper and UPM-Kymmene Kraft (Dube, 2001; Dube *et al.*, 2000a, 2000b, 2000c).

At Irving Paper, NB, a 28-day mummichog exposure test using control receiving water and 3% effluent was conducted, with the following endpoints: survival, growth, gonad and liver size, condition factor, mixed function oxygenase (MFO) induction, reproductive hormone levels, and tracers of effluent exposure. The results indicated significantly reduced gonad weight in males, and increased DHAA levels in the bile of exposed adult males. Effluent exposure did not affect growth.

At Irving Pulp and Paper, NB, two mesocosm studies were conducted. These were a 30-day and a 60-day mummichog exposure test using 5% effluent with the following endpoints: juvenile growth, adult organ size (liver, gonad), condition, MFO induction, and reproductive hormone levels. After the 30-day exposure, liver size had significantly increased among adult females. After 60-day exposure, increased liver size was noted in exposed adults of both sexes, and length and body weight were significantly lower among juvenile mummichogs exposed to the 5% effluent.

At UPM-Kymmene Kraft, NB, a 23-day mummichog exposure test using primary and secondary treated bleached kraft mill effluent at 1% concentration was conducted. The testosterone levels in mummichogs were measured. The results indicated that compounds that depress plasma testosterone levels in mummichogs were present in treated pulp mill effluent.

These studies indicated that mesocosms could be used as an alternative to field fish surveys. Most of the endpoints that are used in the field fish survey can be measured in mesocosm tests. Presently, the EEM fish survey assesses effects using survival, energy use and storage as effect endpoints. In addition to these endpoints, the mesocosm studies measured MFO induction and sex steroid hormones. The results suggest that:

- pulp mill effluent (PME) does not affect survival and growth in a 30-day (or less) exposure;
- PME contains compounds that can alter sex steroid levels in fish; and
- the degree of removal of these compounds from effluent varies depending on the effluent treatment process.

More studies and research are needed to better understand the meaning of the alteration of sex steroid levels and to document the natural variability in sex steroid hormone levels and its effects in mummichogs.

6.2 Mesocosm for Benthic Invertebrate Community Studies

At Fraser, NB, a mesocosm study was conducted to study the effects of effluent on the benthic community. The endpoints measured included: biomass, survival, insect emergence, and dose-response effects of PME on invertebrate population measures. 0, 1, 5, 10, 20, and 40% effluent concentrations were used. The results of the study indicated the following (Culp *et al.*, 2001) :

- PME from this mill increased the productivity of the riverine food web;
- PME did not affect survivorship of mayflies at all concentrations tested (0-40%) and,
- family richness and EPT index were not affected at 1 and 5% effluent concentration

6.3 Caged Bivalve Study

A 90-day caged bivalve study was undertaken at Kimberly-Clark, NS. The study involved placing cages of blue mussels, *Mytilus edulis*, at five locations in Northumberland Strait near the discharge from the pulp mill. The effect endpoints measured were survival and growth. The results indicated that there was no difference in the survival for any of the cages, and that increased growth was observed at the station closest to the outfall (Andrews and Parker, 1999). However, the tissue chemistry did not confirm exposure to effluent, and detailed plume delineation suggested that exposure from the floating plume was minimal (Courtenay *et al.*, in preparation). Even with the limited pulpmill effluent exposure, increased growth was observed at the station closest to the outfall.

6.4 Fathead Minnow Life Cycle Bioassay

At Fraser, NB, 125-day fathead minnow life cycle bioassays were conducted in a flow-through system. The effect endpoints measured were survival, reproduction and secondary sex characteristics. The results indicated the following (Parrott *et al.*, 2000):

- male fish had the appearance of ovipositors (egg laying structures) at 3% effluent and above,
- at 30-100% effluent concentration, the majority of males looked externally like females,
- effluent caused a significant increase in the growth of fish (effect can be seen as little as 30 days)
- change in external sex characteristics required 4 month exposure

- at 1%, some female fish had male sex characteristics
- fish exposed to 1% effluent were the only ones to produce eggs in the study (controls did not).

6.5 Caged Fish Studies

At Fraser, NB, wild white suckers were exposed to effluent for 4 days to investigate biologically active compounds accumulating from effluent. The results indicated that fish rapidly accumulate ligands for Ah receptors and sex steroid receptors from final effluent from the mill. Both the effluent from this mill and the Saint John River water have compounds that elicit MFO induction, and interact with fish sex steroid binding protein receptors. More research and study is required to confirm these results and to understand their ecological relevance and implications (Hewitt *et al.*, 2001).

6.6 Artificial Substrate Samplers (ASS)

ASS were used at 2 mills: Fraser, NB, and Corner Brook Pulp and Paper, NF. (ESG, 2000; Beak International Inc., 2000)

ASS were used to assess the effect of pulp mill effluent on the benthic community. The effect endpoints measured included all the effect endpoints from the standard benthic invertebrate community study (abundance, richness, diversity, and EPT index). ASS were able to eliminate the confounding effects of historical deposition of wood waste and contamination; however, ASS are not recommended unless there are no other alternatives available (EC, 1998).

7.0 CONCLUSION AND RECOMMENDATIONS

A summary of the results for the studies conducted under Cycle 2 EEM for the pulp and paper mills in the Atlantic Region are presented in Table 21.

7.1 Fish Survey

Some differences in condition factor, liver and reproductive endpoints were observed in fish from the exposed area. Whether these observed differences are solely caused by pulp mill effluent is not known. The data could be better assessed with more information on fish movement to confirm duration of exposure. Increased fishing efforts, information on local fish species and its biology, new assessment tools and alternative approaches are needed to assess the effect of pulp mill effluent on fish for the mills that did not conduct a fish study.

Lessons learned from Cycle 2 fish surveys:

7.1.1 Recommendations

- More efforts should be expended in finding appropriate reference sites.
- Multiple reference sites should be considered to assess natural variability in the measured parameters.
- Alternative methods (caged bivalve test, mesocosm studies, other laboratory bioassays) of assessing fish health should be used at sites where there are confounding factors (other effluent discharges, historical contamination).
- The mesocosm studies and long-term life cycle test indicated that the pulp mill effluent contains compounds that can affect the endocrine system. While assessing endocrine disrupting compounds is not part of the current EEM program, more research and studies should be conducted to confirm these observed effects and to understand the implication of these effects in our ecosystem.
- Sedentary small-bodied fish can be used as they are less mobile than large fish which will increase the likelihood of exposure; however, their mobility should be confirmed. Plans are underway to assess the presence and the mobility of mummichog at various sites in the Atlantic Region by EC and DFO.
- Tthree-spine stickleback should not be used as it was difficult to age and extracting bile was difficult for tracer work

7.2 Benthic Invertebrate Survey

The Cycle 2 benthic invertebrate studies concluded 'No Effects' at six sites (33%), and some effects at twelve sites (67%), which is an improvement from Cycle 1, where effects were found at most mills (Table 12).

67% of studies found statistical differences in at least one of the benthic indices (abundance, richness and diversity index), and statistical difference in all three indices was found at 44% (8 of 18) of the mills.

7.2.1 Recommendations

- More effort should be directed at the receiving environment to characterize its physical, chemical and biological features, to assist with the study design and interpretation.

- The use of a simple control/impact study design should be reviewed with respect to addressing natural variability in the measured parameters.

- Detailed guidance on laboratory sub-sampling should be included in the TGD (Environment Canada, 1998). The criteria as to when sub-sampling should be done should be better defined in the TGD (Environment Canada, 1998). The term "large number of organisms" needs to be quantified to achieve consistency from mill to mill.

- Laboratory sub-sampling methodology should be thoroughly documented in all future reports including a plot of the relationship between the total number of individuals counted in the sub-sample and the associated percentage error at a 95% probability level. In the absence of a detailed sub-sampling procedure, a minimum of 100 organisms/sub-sample with variability of <20% has been accepted in this Region, and is recommended for all future work until further guidance is available from the Benthic Invertebrate Expert Working Group.

- Additional guidance on data interpretation for the gradient study design should be included in the TGD (Environment Canada, 1998).

- Alternative studies should be explored at sites where confounding effects are present and where field studies can not be conducted to assess the effects of present pulp mill effluent discharges.

- Multiple reference sites should be used, as recommended by the TGD (Environment Canada, 1998).

- Following confirmation of apparent effects on benthic invertebrates (as judged by statistically significant differences associated with exposure), interpretation of these effects would be aided by more focussed monitoring to investigate the magnitude, geographic extent and cause of effects.

7.3 Effluent Sublethal Toxicity Tests

The effluent quality improved at most mills. In fact, most of the effluents discharging to estuarine/marine environment did not elicit a toxic response in the 7-day fish growth and survival test; however, toxicity is still observed in invertebrate and the algae tests.

The sublethal toxicity tests have met Objective 1, as outlined in Section 5.1, by contributing potential sublethal toxicity data (cause and effect) to the EEM program for each mill as part of the weight of evidence approach. A comparison of the effluent quality between Cycle 1 and Cycle 2 as a result of effluent treatment and process changes was also conducted using the data, satisfying Objective 2. The use of the data to understand the relative contribution of the mill in multiple discharge situations as specified in Objective 3 was not conducted, as Cycle 2 was designed to assess environmental impacts and to determine the magnitude and spatial extent of the impact, if any. However, in future cycles, for the mills where impacts are seen and multiple discharges are present, sublethal toxicity tests will play a key role in determining the relative contribution of the mill.

7.3.1 Work Currently in Progress and Possible Upcoming Changes

The greatest effluent concentration tested in marine tests is 67% due to salinity adjustment. EC is working on a direct salt addition method to increase the testing range of effluent that discharge into estuarine/marine system. This method should be available during Cycle 3.

For the mills with 6 consecutive 'no responses' in any sublethal toxicity test, a consideration **may be** given to reducing the frequency of that test to 1 summer and 1 winter for the next cycle.

A pilot study to assess the relationship between the sublethal toxicity data and the field effects using the data from the Ontario region has been completed.

Fish reproductive and early life stage toxicity tests should be explored, as gonad weight, fecundity and liver weight are the key parameters being affected by pulp mill effluent in fish. Resources should be allocated to fund research related to long-term tests that include these endpoints.

7.4 Research

In Cycle 2, the mills were not required to conduct alternative studies as none were approved for Cycle 2 by the EEM Science Committee. The mills which undertook these alternative studies should be applauded for their commitment to understand the effects of their effluent on the receiving environment. Thanks to their efforts, the EEM Science Committee had additional data (in addition to the work conducted on mesocosms and caged bivalves in Canada and other countries) to evaluate alternative studies for EEM.

The Science Committee has assessed and approved the use of mesocosms and caged bivalves for Cycle 3. The technical guidance documents for these alternatives will be completed in the near future, and will be posted on Environment Canada's EEM website: www.ec.gc.ca/eem

7.4.1 Recommendation

- A summary of research results, including an integration of all studies (field, laboratory and research) conducted for the mill for a given cycle should be presented in the interpretation reports.

- Furthermore it should be noted that the mesocosm studies, long term life-cycle test and the caged fish test all indicated that the pulp mill effluent contained compounds that could affect the endocrine system. While the study of endocrine disrupters are outside the current EEM program, more research and studies should be conducted to confirm these observed effects and to understand the implication of these effects in our ecosystem.

Table 21. Summary of Cycle 2 EEM Study Results in the Atlantic Region

Mills	Toxicity IC25 (geometric mean %)	Fish Study (observations in exposed fish when compared to fish from reference areas)	Benthic Invertebrate Community Study	Location of the benthic sampling station with respect to the Distance from Outfall
Freshwater				
Abitibi-Consolidated Grand Falls	#3 Sewer/ASB/N.Sewer P: 60.4/64.9/73.2 D: 15.94/39.04/29.85 S: 50.4/34.6/71.4	N/A - 1% plume <250 m	Total abundance (ES=8.8SD), and # of taxa (ES=1.8SD) were higher in the exposure area: moderate nutrient enrichment.	Exposure Area: approx. 2.5 km from North Sewer
Fraser	P: 42.8 D: 16.75 S: 65.1	**Research-** **125-day Fathead Minnow Life Cycle Test:** • At 3% effluent exposure, male fish had the appearance of ovipositors. • At 30-100% effluent concentration, the majority of males looked externally like females. • At 4-month effluent exposure, a change in external sex characteristics observed. • At 1% effluent exposure, some females had male sex characteristics. • Increase in growth seen as little as 30 days.	**Surber Study** - Compared with R2 . Statistical difference in total abundance and EPT index in NF and FF. Statistical difference in Shannon. D. index in NF and statistical difference in richness in FF. **Artificial Substrate Study**: no effect. **Research - Mesocosm Study:** • Family richness and EPT index were not affected at 1 and 5% effluent concentration • PME did not affect survivorship of mayflies (0-40%). • PME increased the productivity of the riverine food web.	NF: 0.5 - 2.5 km FF: 11-15 km
St. Anne-Nackawic	P: 100 D: 29.26 S: 38.7	Yellow Perch: • M shorter, lower gonad wt., lighter, smaller liver • F shorter, 40% lighter, smaller gonads & eggs, lighter liver	Increased abundance in NF Shallow area (>2SD); decrease abundance at FF Deep area (<2SD). Richness higher in NF of Deep area (<2SD).	NF: within 0.5 km FF: 2 - 2.7 km

Table 21. Summary of Cycle 2 EEM Study Results in the Atlantic Region (continued)

Mills	Toxicity IC25 (geometric mean %)	Fish Study Effects in the Exposure Area	Benthic Invertebrate Community Study	Location of the benthic sampling station with respect to the Distance from Outfall
Estuarine/ Marine				
Abitibi-Consolidated Stephenville	M: >67 L: 25.77 C: 7.88	N/A - rapid dilution	No statistically significant effect observed, **but** increase in abundance and decrease in total number of taxa in NF, and increase in abundance and taxa in the North FF area observed.	24-1065 m
AV Cell	M: 63.31 L: 3.45 C: 5.55	**Sticklebacks:** • More females M: increase in liver weight, F: decrease in gonad and total weight, smaller eggs **Mummichogs:** M: older, higher condition F: slightly younger, heavier, 21-29% more eggs in a 'standarized' fish from exposure area (see text).	Statistical difference in all indices, **but** subsequent testing indicated no pattern with the distance from outfall. Increased abundance of Paranais and Chironomous in NF observed. Reference area questionable.	U/S Gradient: 50-800 m D/S Gradient: 50-2400 m
Bowater Maritimes	M: >67 L: 27.81 C: 11.51	N/A - reconnaisance survey	No statistical difference in total abundance, richness and diversity indices; **however,** significant difference in polychaete abundance observed for NF when compared to R2.	NF: within 300 metres FF: not sampled
Bowater Mersey	M: >67 L: 35.21 C: 14.89	Could not catch enough fish to perform an AFS	Increased abundance, decreased richness and diversity in exposure area. Historical woodwaste confounded data interpretation.	NF: approx. 200 m FF: approx. 750 m FFF: approx. 1500 m

Table 21. Summary of Cycle 2 EEM Study Results in the Atlantic Region (continued)

Mills	Toxicity IC25 (geometric mean %)	Fish Study	Benthic Invertebrate Community Study	Location of the benthic sampling station with respect to the Distance from Outfall
CKF/Minas Basin	M: >67 L: 61.66 C: 16.33	N/A - 1% plume <250 m	No statistical difference was observed when the number of taxa for Transect 2 was regressed according to distance. A reduction in abundance and number of taxa with increasing distance from outfall was observed; however, TOC, sulfide and redox measurements did not support nutrient enrichment effect. Salinity gradient present.	0-200 m (north and south)
Corner Brook Pulp and Paper	M: >67 L: 61.15 C: 46.06	Fish Study Not Done – Confounding Factors	No statistically significant effects **but** decreased abundance and mean number of taxa are observed in the NF and FF. FFW dominated by Capitella.	NF: approx. 50 m FF: approx. 200 m FFW: approx. 200 m
Irving Paper	M: >67 L: 42.06 C: 17.29	**Research – Mesocosm Study using mummichogs** • 28-day 3% Effluent exposure • Significantly reduced gonad weight in both sexes • Significant increase in DHAA found in bile of exposed males • liver weight unaffected	Low abundance, number of species, diversity and biomass in NF area.	NF: approx. 250 m
Irving Pulp and Paper	M: 35.87 L: 13.44 C: 8.63	**Research – Mesocosm Study using Mummichogs** • 30-day and 60-day exposure to 5% effluent • After 30-day exposure, liver size significantly increased in exposed adult females • After 60 days, liver size significantly increased among exposed adults of both sexes • After 60 days, total body weight and length of juveniles significantly decreased	No statistical effect when indices were regressed over distance from outfall; **however,** diversity and evenness decreased with increasing distance from mill effluent outfall: mild nutrient enrichment.	0-5.6 km

Table 21. Summary of Cycle 2 EEM Study Results in the Atlantic Region (continued)

Mills	Toxicity IC25 (geometric mean %)	Fish Study	Benthic Invertebrate Community Study	Location of the benthic sampling station with respect to the Distance from Outfall
Kimberly-Clark	M: >67 L: 5.13 C: 5.47	**Research – Caged Bivalve Study** • No effect; however, increased growth observed at the site closest to the outfall.	No statistical difference in total abundance, richness and diversity; **however,** high polychaete density in FF (p=0.015). Nutrient enrichment effect in Intertidal area.	NF: approx. 500 m FF: approx. 1000 m
L.P. East River	M: >67 L: 4.23 C: 6.49	N/A - 1% < 250 m	Decrease in abundance, richness and diversity in the NF. Extent of impact <200 m.	NF: 37 - 96 m FF: 129 m
Lake Utopia	M: 55.54 L: 0.89 C: 1.71	N/A - Fish Reconnaissance Survey	**Impoundment**: All samples had low abundance, number of species and diversity. There was no relationship with benthic indices and the distance from the outfall. All the stations in the impoundment were affected. The impoundment has layers of historical deposit of woodwaste. **Estuary**: Abundance, number of taxa and species diversity increased with increasing distance from the outfall.	Imp: 0-3.25 km Est: 3.5-5.45 km
Smurfit Stone Container	M: >67 L: 1.76 C: 0.91	**Sticklebacks**: • M decrease in age, and body weight; increase in liver weight • F decrease in age, body weight, and gonad weight; increase in liver weight **Mummichogs:** • M decrease in age, • F decrease in age; increase in liver weight	Decreased abundance, richness and diversity in NF (ES >2SD). NF comprised of pollution tollerant taxa.	C/I: up to 400 m Gradient: <100 to 4 km

Table 21. Summary of Cycle 2 EEM Study Results in the Atlantic Region (continued)

Mills	Toxicity IC25 (geometric mean %)	Fish Study	Benthic Invertebrate Community Study	Location of the benthic sampling station with respect to the Distance from Outfall
Stora Enso	M: >67 L: 4.93 C: 6.52	**Winter Flounder:** • M body slightly smaller in size, larger livers • F younger (only 2 sexually mature female flounders were caught)	Abundance in FF statistically different from RB. Number of Taxa in all the exposure areas is statistically different from RB. Diversity in NF statistically different from RA.	NF: <100 m FF: approx. 500 m
UPM-Kymmene Groundwood	M: >67 L: 40.01 C: 14.49	N/A - 1% plume <250 m	Low abundance, richness and diversity at far-field (station G6) and far-far-field (station G4). Effect confounded by salinity/tidal effect.	NF: approx. 125-225 m FF: approx. 550-575 m
UPM-Kymmene Kraft	M: 66 L: 2.93 C: 2.71	**Mummichogs:** • A decrease in the number of eggs in exposed female. **Research – Mesocosm Study using Mummichgos** • 23-day exosure to 1% primary-treated effluent and 1% secondary-treated effluent • plasma testosterone levels significantly decreased among both sexes, for both primary- and seconday-treated effluent.	Decreased abundance, species richness and diversity in the NF in deep water. Effect confounded by salinity/tidal effect.	NF-shallow: approx.185-440 m NF-deep: approx. 160-825 m

P=*Pimephales promelas* ; D=*Ceriodaphnia dubia*; S=*Selanastrum capricornutum*: M=*Menidia beryllina*; L=*Lytechinus pictus*; C=*Champia parvula*

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Appendix 1. The Cycle 2 EEM Technical Advisory Panels (TAP) in the Atlantic Region

Province	TAP Chair	TAP Members	Affiliation
New Brunswick TAP	George Lindsay/ Roy Parker (EC)	Hélène Dupuis	DFO, Oceans Branch
		Simon Courtenay	DFO, Ocean Branch
		Don Murray	NBDOELG
		Mike Cormier	NBDOELG
		Kelly Munkittrick	EC (now with UNB)
		Victor Li/M.T. Grant	EC
		Kay Kim	EC
Nova Scotia TAP	Roy Parker/ Kay Kim (EC)	Hélène Dupuis	DFO, Oceans Branch
		Simon Courtenay	DFO, Oceans Branch
		Kate Moir	NSDOE
		Kelly Munkittrick	EC (now with UNB)
		Victor Li/M.T. Grant	EC
Newfoundland TAP	Roy Parker/ Kay Kim (EC)	Stephen Snow	DFO
		Brenda Brown	DFO
		Derrick Maddocks	NFDEL
		Kevin Power	EC
		Kim Coady/ Shauna Pierce	EC
EEM Coordinator for Cycle 2	Roy Parker/Kay Kim (EC)		

EC = Environment Canada
DFO = Department of Fisheries and Oceans
NBDOELG = New Brunswick Department of Environment and Local Government
UNB = University of New Brunswick
NSDOE = Nova Scotia Department of Environment
NFDEL = Newfoundland Department of Environment and Labour

Appendix 2. IC_{25} for the Sublethal Toxicity Tests conducted in Cycle 2 in the Atlantic Region (April 1996-April 2000)

Mill	Test Date (YYYYMMDD)	Species Tested	EC_{25} or IC_{25} %
Abitibi-Consolidated Grand Falls (#3 Sewer)	19971027	*Ceriodaphnia dubia*	8.80
Abitibi-Consolidated Grand Falls (#3 Sewer)	19980330	*Ceriodaphnia dubia*	12.30
Abitibi-Consolidated Grand Falls (#3 Sewer)	19991007	*Ceriodaphnia dubia*	22.00
Abitibi-Consolidated Grand Falls (#3 Sewer)	19991214	*Ceriodaphnia dubia*	8.00
Abitibi-Consolidated Grand Falls (#3 Sewer)	20000304	*Ceriodaphnia dubia*	54.10
Abitibi-Consolidated Grand Falls (#3 Sewer)	19971027	*Pimephales promelas*	80.20
Abitibi-Consolidated Grand Falls (#3 Sewer)	19980330	*Pimephales promelas*	33.80
Abitibi-Consolidated Grand Falls (#3 Sewer)	19991007	*Pimephales promelas*	72.60
Abitibi-Consolidated Grand Falls (#3 Sewer)	19991214	*Pimephales promelas*	57.80
Abitibi-Consolidated Grand Falls (#3 Sewer)	20000304	*Pimephales promelas*	70.60
Abitibi-Consolidated Grand Falls (#3 Sewer)	19971027	*Selanastrum capricomutum*	100.00
Abitibi-Consolidated Grand Falls (#3 Sewer)	19980330	*Selanastrum capricomutum*	54.40
Abitibi-Consolidated Grand Falls (#3 Sewer)	19991007	*Selanastrum capricomutum*	16.60
Abitibi-Consolidated Grand Falls (#3 Sewer)	19991214	*Selanastrum capricomutum*	100.00
Abitibi-Consolidated Grand Falls (#3 Sewer)	20000304	*Selanastrum capricomutum*	36.10
Abitibi-Consolidated Grand Falls (ASB)	19971027	*Ceriodaphnia dubia*	49.8
Abitibi-Consolidated Grand Falls (ASB)	19980330	*Ceriodaphnia dubia*	35.6
Abitibi-Consolidated Grand Falls (ASB)	19991007	*Ceriodaphnia dubia*	48.7
Abitibi-Consolidated Grand Falls (ASB)	19991214	*Ceriodaphnia dubia*	26.9
Abitibi-Consolidated Grand Falls (ASB)	20000304	*Ceriodaphnia dubia*	NA
Abitibi-Consolidated Grand Falls (ASB)	19971027	*Pimephales promelas*	100.00
Abitibi-Consolidated Grand Falls (ASB)	19980330	*Pimephales promelas*	55.4
Abitibi-Consolidated Grand Falls (ASB)	19991007	*Pimephales promelas*	100.00
Abitibi-Consolidated Grand Falls (ASB)	19991214	*Pimephales promelas*	25.0
Abitibi-Consolidated Grand Falls (ASB)	20000304	*Pimephales promelas*	83.40
Abitibi-Consolidated Grand Falls (ASB)	19971027	*Selanastrum capricomutum*	100.00
Abitibi-Consolidated Grand Falls (ASB)	19980330	*Selanastrum capricomutum*	100.00
Abitibi-Consolidated Grand Falls (ASB)	19991007	*Selanastrum capricomutum*	3.90
Abitibi-Consolidated Grand Falls (ASB)	19991214	*Selanastrum capricomutum*	36.80
Abitibi-Consolidated Grand Falls (ASB)	20000304	*Selanastrum capricomutum*	N/A
Abitibi-Consolidated Grand Falls (North Sewer)	19971027	*Ceriodaphnia dubia*	13.30
Abitibi-Consolidated Grand Falls (North Sewer)	19980330	*Ceriodaphnia dubia*	11.00
Abitibi-Consolidated Grand Falls (North Sewer)	19991007	*Ceriodaphnia dubia*	16.20
Abitibi-Consolidated Grand Falls (North Sewer)	19991214	*Ceriodaphnia dubia*	100.00
Abitibi-Consolidated Grand Falls (North Sewer)	20000304	*Ceriodaphnia dubia*	100.00
Abitibi-Consolidated Grand Falls (North Sewer)	19971027	*Pimephales promelas*	68.60
Abitibi-Consolidated Grand Falls (North Sewer)	19980330	*Pimephales promelas*	77.10
Abitibi-Consolidated Grand Falls (North Sewer)	19991007	*Pimephales promelas*	39.60
Abitibi-Consolidated Grand Falls (North Sewer)	19991214	*Pimephales promelas*	100.00
Abitibi-Consolidated Grand Falls (North Sewer)	20000304	*Pimephales promelas*	100.00
Abitibi-Consolidated Grand Falls (North Sewer)	19971027	*Selanastrum capricomutum*	100.00
Abitibi-Consolidated Grand Falls (North Sewer)	19980330	*Selanastrum capricomutum*	100.00
Abitibi-Consolidated Grand Falls (North Sewer)	19991007	*Selanastrum capricomutum*	18.60

Appendix 2. IC_{25} for the Sublethal Toxicity Tests conducted in Cycle 2 in the Atlantic Region (April 1996-April 2000)

Mill	Test Date (YYYYMMDD)	Species Tested	EC_{25} or IC_{25} %
Abitibi-Consolidated Grand Falls (North Sewer)	19991214	*Selanastrum capricornutum*	100.00
Abitibi-Consolidated Grand Falls (North Sewer)	20000304	*Selanastrum capricornutum*	100.00
Abitibi-Consolidated Stephenville	19971028	*Champia parvula*	9.64
Abitibi-Consolidated Stephenville	19980413	*Champia parvula*	33.54
Abitibi-Consolidated Stephenville	19990331	*Champia parvula*	8.63
Abitibi-Consolidated Stephenville	19991006	*Champia parvula*	1.77
Abitibi-Consolidated Stephenville	20000329	*Champia parvula*	6.17
Abitibi-Consolidated Stephenville	19971028	*Lytechinus pictus*	34.6
Abitibi-Consolidated Stephenville	19980413	*Lytechinus pictus*	28.7
Abitibi-Consolidated Stephenville	19990331	*Lytechinus pictus*	43.6
Abitibi-Consolidated Stephenville	19991006	*Lytechinus pictus*	28.9
Abitibi-Consolidated Stephenville	20000329	*Lytechinus pictus*	9.08
Abitibi-Consolidated Stephenville	19971028	*Menidia beryllina*	>72
Abitibi-Consolidated Stephenville	19980413	*Menidia beryllina*	>71
Abitibi-Consolidated Stephenville	19990331	*Menidia beryllina*	>67
Abitibi-Consolidated Stephenville	19991006	*Menidia beryllina*	>67
Abitibi-Consolidated Stephenville	20000329	*Menidia beryllina*	>68
AV Cell	19990222	*Champia parvula*	3.08
AV Cell	19991019	*Champia parvula*	13.81
AV Cell	20000327	*Champia parvula*	4.01
AV Cell	19990222	*Lytechinus pictus*	2.6
AV Cell	19991019	*Lytechinus pictus*	3.48
AV Cell	20000327	*Lytechinus pictus*	4.53
AV Cell	19990222	*Menidia beryllina*	60.3
AV Cell	19991019	*Menidia beryllina*	62.8
AV Cell	20000327	*Menidia beryllina*	>67
Bowater Maritimes	19970929	*Champia parvula*	0.24
Bowater Maritimes	19980325	*Champia parvula*	50.4
Bowater Maritimes	19980914	*Champia parvula*	>68.1
Bowater Maritimes	19990309	*Champia parvula*	16.7
Bowater Maritimes	19990927	*Champia parvula*	12.7
Bowater Maritimes	20000208	*Champia parvula*	13.3
Bowater Maritimes	19970916	*Lytechinus pictus*	47.1
Bowater Maritimes	19980413	*Lytechinus pictus*	20.1
Bowater Maritimes	19980914	*Lytechinus pictus*	9.77
Bowater Maritimes	19990309	*Lytechinus pictus*	38.1
Bowater Maritimes	19990927	*Lytechinus pictus*	32.3
Bowater Maritimes	20000208	*Lytechinus pictus*	40.6
Bowater Maritimes	19970916	*Menidia beryllina*	>72
Bowater Maritimes	19980325	*Menidia beryllina*	>70
Bowater Maritimes	19980914	*Menidia beryllina*	>67
Bowater Maritimes	19990309	*Menidia beryllina*	>67
Bowater Maritimes	19990927	*Menidia beryllina*	>67

Appendix 2. IC_{25} for the Sublethal Toxicity Tests conducted in Cycle 2 in the Atlantic Region (April 1996-April 2000)

Mill	Test Date (YYYYMMDD)	Species Tested	EC_{25} or IC_{25} %
Bowater Maritimes	20000208	*Menidia beryllina*	>68
Bowater Mersey	19970903	*Champia parvula*	7.10
Bowater Mersey	19980309	*Champia parvula*	33.6
Bowater Mersey	19980817	*Champia parvula*	15.0
Bowater Mersey	19990223	*Champia parvula*	7.12
Bowater Mersey	19990907	*Champia parvula*	11.0
Bowater Mersey	20000207	*Champia parvula*	38.9
Bowater Mersey	19970903	*Lytechinus pictus*	60.00
Bowater Mersey	19980309	*Lytechinus pictus*	7.39
Bowater Mersey	19980817	*Lytechinus pictus*	27.8
Bowater Mersey	19990223	*Lytechinus pictus*	>67
Bowater Mersey	19990907	*Lytechinus pictus*	38.3
Bowater Mersey	20000207	*Lytechinus pictus*	60.2
Bowater Mersey	19970903	*Menidia beryllina*	>67
Bowater Mersey	19980309	*Menidia beryllina*	>73
Bowater Mersey	19980817	*Menidia beryllina*	>67
Bowater Mersey	19990223	*Menidia beryllina*	>67
Bowater Mersey	19990907	*Menidia beryllina*	>67
Bowater Mersey	20000207	*Menidia beryllina*	>67
CKF/Minas Basin	19971021	*Champia parvula*	27.05
CKF/Minas Basin	19980408	*Champia parvula*	18.94
CKF/Minas Basin	19980818	*Champia parvula*	11.49
CKF/Minas Basin	19990330	*Champia parvula*	4.97
CKF/Minas Basin	19990921	*Champia parvula*	31.12
CKF/Minas Basin	20000314	*Champia parvula*	20.80
CKF/Minas Basin	19971021	*Lytechinus pictus*	>71
CKF/Minas Basin	19980408	*Lytechinus pictus*	58.6
CKF/Minas Basin	19980818	*Lytechinus pictus*	42
CKF/Minas Basin	19990318	*Lytechinus pictus*	>67
CKF/Minas Basin	19990824	*Lytechinus pictus*	>68
CKF/Minas Basin	20000314	*Lytechinus pictus*	>69
CKF/Minas Basin	19971021	*Menidia beryllina*	>71
CKF/Minas Basin	19980408	*Menidia beryllina*	>73
CKF/Minas Basin	19980818	*Menidia beryllina*	>67
CKF/Minas Basin	19990318	*Menidia beryllina*	>67
CKF/Minas Basin	19990824	*Menidia beryllina*	>67
CKF/Minas Basin	20000314	*Menidia beryllina*	>67
Corner Brook Pulp and Paper	19970812	*Champia parvula*	23.7
Corner Brook Pulp and Paper	19980421	*Champia parvula*	49.1
Corner Brook Pulp and Paper	19980915	*Champia parvula*	>68.7
Corner Brook Pulp and Paper	19990315	*Champia parvula*	>67.3
Corner Brook Pulp and Paper	19991012	*Champia parvula*	44.4
Corner Brook Pulp and Paper	20000207	*Champia parvula*	39.9

Appendix 2. IC_{25} for the Sublethal Toxicity Tests conducted in Cycle 2 in the Atlantic Region (April 1996-April 2000)

Mill	Test Date (YYYYMMDD)	Species Tested	EC_{25} or IC_{25} %
Corner Brook Pulp and Paper	19970929	*Lytechinus pictus*	>67
Corner Brook Pulp and Paper	19980421	*Lytechinus pictus*	>70
Corner Brook Pulp and Paper	19980915	*Lytechinus pictus*	61.0
Corner Brook Pulp and Paper	19990315	*Lytechinus pictus*	40.1
Corner Brook Pulp and Paper	19991012	*Lytechinus pictus*	>67
Corner Brook Pulp and Paper	20000207	*Lytechinus pictus*	>68
Corner Brook Pulp and Paper	19970929	*Menidia beryllina*	>67
Corner Brook Pulp and Paper	19980421	*Menidia beryllina*	>71
Corner Brook Pulp and Paper	19980915	*Menidia beryllina*	>67
Corner Brook Pulp and Paper	19990315	*Menidia beryllina*	>67
Corner Brook Pulp and Paper	19991012	*Menidia beryllina*	>68
Corner Brook Pulp and Paper	20000207	*Menidia beryllina*	>68
Fraser	19970924	*Ceriodaphnia dubia*	33.6
Fraser	19980403	*Ceriodaphnia dubia*	18.3
Fraser	19981030	*Ceriodaphnia dubia*	22.2
Fraser	19990406	*Ceriodaphnia dubia*	32.8
Fraser	19991018	*Ceriodaphnia dubia*	26.0
Fraser	20000208	*Ceriodaphnia dubia*	1.90
Fraser	19970924	*Pimephales promelas*	59.6
Fraser	19980403	*Pimephales promelas*	33.8
Fraser	19981030	*Pimephales promelas*	39.2
Fraser	19990406	*Pimephales promelas*	45
Fraser	19991018	*Pimephales promelas*	39.2
Fraser	20000207	*Pimephales promelas*	44.00
Fraser	19970926	*Selenastrum capricornutum*	80.9
Fraser	19980402	*Selenastrum capricornutum*	42.8
Fraser	19981001	*Selenastrum capricornutum*	>100
Fraser	19990408	*Selenastrum capricornutum*	41.0
Fraser	19991018	*Selenastrum capricornutum*	53.5
Fraser	20000207	*Selenastrum capricornutum*	>100
Irving Pulp and Paper	19970929	*Champia parvula*	5.45
Irving Pulp and Paper	19980427	*Champia parvula*	1.36
Irving Pulp and Paper	19981026	*Champia parvula*	13.63
Irving Pulp and Paper	19990303	*Champia parvula*	12.01
Irving Pulp and Paper	19991020	*Champia parvula*	33.5
Irving Pulp and Paper	20000419	*Champia parvula*	10.20
Irving Pulp and Paper	19970929	*Lytechinus pictus*	4.84
Irving Pulp and Paper	19980423	*Lytechinus pictus*	12.1
Irving Pulp and Paper	19981026	*Lytechinus pictus*	20.2
Irving Pulp and Paper	19990303	*Lytechinus pictus*	19.3
Irving Pulp and Paper	19991020	*Lytechinus pictus*	14.1
Irving Pulp and Paper	20000419	*Lytechinus pictus*	18.30
Irving Pulp and Paper	19970929	*Menidia beryllina*	>66.6

Appendix 2. IC_{25} for the Sublethal Toxicity Tests conducted in Cycle 2 in the Atlantic Region (April 1996-April 2000)

Mill	Test Date (YYYYMMDD)	Species Tested	EC_{25} or IC_{25} %
Irving Pulp and Paper	19980423	*Menidia beryllina*	2.2
Irving Pulp and Paper	19981026	*Menidia beryllina*	>67
Irving Pulp and Paper	19990303	*Menidia beryllina*	>67
Irving Pulp and Paper	19991020	*Menidia beryllina*	48.3
Irving Pulp and Paper	20000419	*Menidia beryllina*	>67
Irving Paper	19970929	*Champia parvula*	9.24
Irving Paper	19980420	*Champia parvula*	21.2
Irving Paper	19981013	*Champia parvula*	18.0
Irving Paper	19990420	*Champia parvula*	63.4
Irving Paper	19991013	*Champia parvula*	2.13
Irving Paper	20000418	*Champia parvula*	56.20
Irving Paper	19970929	*Lytechinus pictus*	>66.6
Irving Paper	19980420	*Lytechinus pictus*	45.0
Irving Paper	19981013	*Lytechinus pictus*	>67.0
Irving Paper	19990420	*Lytechinus pictus*	>67
Irving Paper	19991013	*Lytechinus pictus*	10.5
Irving Paper	20000418	*Lytechinus pictus*	39.20
Irving Paper	19970929	*Menidia beryllina*	>66
Irving Paper	19980420	*Menidia beryllina*	>71
Irving Paper	19981013	*Menidia beryllina*	>67
Irving Paper	19990420	*Menidia beryllina*	>67
Irving Paper	19991013	*Menidia beryllina*	>67
Irving Paper	20000418	*Menidia beryllina*	>70
Kimberly Clark NS	19971021	*Champia parvula*	2.87
Kimberly Clark NS	19980414	*Champia parvula*	13.2
Kimberly Clark NS	19980804	*Champia parvula*	3.97
Kimberly Clark NS	19990412	*Champia parvula*	5.00
Kimberly Clark NS	19990914	*Champia parvula*	5.99
Kimberly Clark NS	20000125	*Champia parvula*	5.97
Kimberly Clark NS	19971021	*Lytechinus pictus*	3.84
Kimberly Clark NS	19980414	*Lytechinus pictus*	7.92
Kimberly Clark NS	19980804	*Lytechinus pictus*	4.30
Kimberly Clark NS	19990412	*Lytechinus pictus*	6.33
Kimberly Clark NS	19990914	*Lytechinus pictus*	6.06
Kimberly Clark NS	20000125	*Lytechinus pictus*	3.63
Kimberly Clark NS	19971021	*Menidia beryllina*	>72
Kimberly Clark NS	19980414	*Menidia beryllina*	>71
Kimberly Clark NS	19980804	*Menidia beryllina*	>67
Kimberly Clark NS	19990412	*Menidia beryllina*	>67
Kimberly Clark NS	19990914	*Menidia beryllina*	>67
Kimberly Clark NS	20000125	*Menidia beryllina*	>71
L.P. East River	19970923	*Champia parvula*	0.469
L.P. East River	19980323	*Champia parvula*	29.92

Appendix 2. IC_{25} for the Sublethal Toxicity Tests conducted in Cycle 2 in the Atlantic Region (April 1996-April 2000)

Mill	Test Date (YYYYMMDD)	Species Tested	EC_{25} or IC_{25} %
L.P. East River	19980824	*Champia parvula*	31.4
L.P. East River	19990323	*Champia parvula*	5.22
L.P. East River	19991012	*Champia parvula*	4.13
L.P. East River	20000314	*Champia parvula*	7.85
L.P. East River	19970923	*Lytechinus pictus*	4.93
L.P. East River	19980323	*Lytechinus pictus*	2.27
L.P. East River	19980824	*Lytechinus pictus*	4.61
L.P. East River	19990412	*Lytechinus pictus*	4.82
L.P. East River	19991012	*Lytechinus pictus*	13.2
L.P. East River	20000314	*Lytechinus pictus*	1.75
L.P. East River	19970923	*Menidia beryllina*	>71
L.P. East River	19980323	*Menidia beryllina*	>70
L.P. East River	19980824	*Menidia beryllina*	>67
L.P. East River	19990412	*Menidia beryllina*	>67
L.P. East River	19991012	*Menidia beryllina*	>70
L.P. East River	20000314	*Menidia beryllina*	>67
Lake Utopia	19970929	*Champia parvula*	0.45
Lake Utopia	19980420	*Champia parvula*	3.17
Lake Utopia	19981013	*Champia parvula*	4.96
Lake Utopia	19990420	*Champia parvula*	1.81
Lake Utopia	19991003	*Champia parvula*	0.77
Lake Utopia	20000419	*Champia parvula*	2.61
Lake Utopia	19970929	*Lytechinus pictus*	0.37
Lake Utopia	19980420	*Lytechinus pictus*	0.42
Lake Utopia	19981013	*Lytechinus pictus*	4.92
Lake Utopia	19990420	*Lytechinus pictus*	0.26
Lake Utopia	19991003	*Lytechinus pictus*	1.56
Lake Utopia	20000419	*Lytechinus pictus*	1.63
Lake Utopia	19970929	*Menidia beryllina*	>66.6
Lake Utopia	19980420	*Menidia beryllina*	>71
Lake Utopia	19981013	*Menidia beryllina*	>66.9
Lake Utopia	19990420	*Menidia beryllina*	>67
Lake Utopia	19991003	*Menidia beryllina*	19.80
Lake Utopia	20000419	*Menidia beryllina*	>70
St. Anne-Nackawic	19970916	*Ceriodaphnia dubia*	31
St. Anne-Nackawic	19980323	*Ceriodaphnia dubia*	13.1
St. Anne-Nackawic	19981005	*Ceriodaphnia dubia*	60.7
St. Anne-Nackawic	19990412	*Ceriodaphnia dubia*	29.0
St. Anne-Nackawic	19990927	*Ceriodaphnia dubia*	42.8
St. Anne-Nackawic	20000412	*Ceriodaphnia dubia*	20.50
St. Anne-Nackawic	19970916	*Pimephales promelas*	>100
St. Anne-Nackawic	19980323	*Pimephales promelas*	>100
St. Anne-Nackawic	19981005	*Pimephales promelas*	>100

Appendix 2. IC_{25} for the Sublethal Toxicity Tests conducted in Cycle 2 in the Atlantic Region (April 1996-April 2000)

Mill	Test Date (YYYYMMDD)	Species Tested	EC_{25} or IC_{25} %
St. Anne-Nackawic	19990412	*Pimephales promelas*	>100
St. Anne-Nackawic	19990927	*Pimephales promelas*	>100
St. Anne-Nackawic	20000412	*Pimephales promelas*	>100
St. Anne-Nackawic	19970916	*Selenastrum capricornutum*	>100
St. Anne-Nackawic	19980323	*Selenastrum capricornutum*	12.9
St. Anne-Nackawic	19981005	*Selenastrum capricornutum*	>100
St. Anne-Nackawic	19990412	*Selenastrum capricornutum*	15.3
St. Anne-Nackawic	19990927	*Selenastrum capricornutum*	>100
St. Anne-Nackawic	20000412	*Selenastrum capricornutum*	17.00
Smurfit Stone Container	19970923	*Champia parvula*	0.22
Smurfit Stone Container	19980302	*Champia parvula*	3.77
Smurfit Stone Container	19980824	*Champia parvula*	5.02
Smurfit Stone Container	19990420	*Champia parvula*	0.45
Smurfit Stone Container	19990928	*Champia parvula*	0.38
Smurfit Stone Container	20000124	*Champia parvula*	0.78
Smurfit Stone Container	19970923	*Lytechinus pictus*	5.60
Smurfit Stone Container	19980203	*Lytechinus pictus*	1.05
Smurfit Stone Container	19980824	*Lytechinus pictus*	2.86
Smurfit Stone Container	19990330	*Lytechinus pictus*	0.66
Smurfit Stone Container	19991012	*Lytechinus pictus*	2.34
Smurfit Stone Container	20000124	*Lytechinus pictus*	1.14
Smurfit Stone Container	19970923	*Menidia beryllina*	>71
Smurfit Stone Container	19980302	*Menidia beryllina*	>73
Smurfit Stone Container	19980824	*Menidia beryllina*	>67
Smurfit Stone Container	19990330	*Menidia beryllina*	>67
Smurfit Stone Container	19990928	*Menidia beryllina*	>67
Smurfit Stone Container	20000124	*Menidia beryllina*	>69
Stora Enso	19970923	*Champia parvula*	0.24
Stora Enso	19980323	*Champia parvula*	7.90
Stora Enso	19980825	*Champia parvula*	>70.1
Stora Enso	19990223	*Champia parvula*	7.89
Stora Enso	19990921	*Champia parvula*	6.88
Stora Enso	20000125	*Champia parvula*	10.6
Stora Enso	19970923	*Lytechinus pictus*	1.1
Stora Enso	19980323	*Lytechinus pictus*	0.65
Stora Enso	19980825	*Lytechinus pictus*	17.0
Stora Enso	19990223	*Lytechinus pictus*	19.0
Stora Enso	19990823	*Lytechinus pictus*	3.42
Stora Enso	20000125	*Lytechinus pictus*	18.3
Stora Enso	19970923	*Menidia beryllina*	>71
Stora Enso	19980323	*Menidia beryllina*	>70
Stora Enso	19980825	*Menidia beryllina*	>67
Stora Enso	19990223	*Menidia beryllina*	>67

Appendix 2. IC_{25} for the Sublethal Toxicity Tests conducted in Cycle 2 in the Atlantic Region (April 1996-April 2000)

Mill	Test Date (YYYYMMDD)	Species Tested	EC_{25} or IC_{25} %
Stora Enso	19990823	*Menidia beryllina*	>67
Stora Enso	20000125	*Menidia beryllina*	>67
UPM-Kymmene Groundwood	19970916	*Champia parvula*	0.56
UPM-Kymmene Groundwood	19980407	*Champia parvula*	>68.6
UPM-Kymmene Groundwood	19980810	*Champia parvula*	>71.92
UPM-Kymmene Groundwood	19990321	*Champia parvula*	9.9
UPM-Kymmene Groundwood	19991011	*Champia parvula*	40
UPM-Kymmene Groundwood	20000224	*Champia parvula*	8.45
UPM-Kymmene Groundwood	19970916	*Lytechinus pictus*	66.4
UPM-Kymmene Groundwood	19980407	*Lytechinus pictus*	>72
UPM-Kymmene Groundwood	19980810	*Lytechinus pictus*	17.2
UPM-Kymmene Groundwood	19990321	*Lytechinus pictus*	35.3
UPM-Kymmene Groundwood	19991011	*Lytechinus pictus*	57
UPM-Kymmene Groundwood	20000224	*Lytechinus pictus*	24.80
UPM-Kymmene Groundwood	19970916	*Menidia beryllina*	>72
UPM-Kymmene Groundwood	19980407	*Menidia beryllina*	60.5
UPM-Kymmene Groundwood	19980810	*Menidia beryllina*	>67
UPM-Kymmene Groundwood	19990321	*Menidia beryllina*	>67
UPM-Kymmene Groundwood	19991011	*Menidia beryllina*	>67
UPM-Kymmene Groundwood	20000224	*Menidia beryllina*	>69
UPM-Kymmene Kraft	19970916	*Champia parvula*	0.19
UPM-Kymmene Kraft	19980407	*Champia parvula*	7.95
UPM-Kymmene Kraft	19980810	*Champia parvula*	7.96
UPM-Kymmene Kraft	19990321	*Champia parvula*	2.56
UPM-Kymmene Kraft	19990906	*Champia parvula*	1.51
UPM-Kymmene Kraft	20000220	*Champia parvula*	8.53
UPM-Kymmene Kraft	19970916	*Lytechinus pictusor*	9.5
UPM-Kymmene Kraft	19980407	*Lytechinus pictusor*	6.0
UPM-Kymmene Kraft	19980810	*Lytechinus pictusor*	0.52
UPM-Kymmene Kraft	19990321	*Lytechinus pictusor*	1.57
UPM-Kymmene Kraft	19990906	*Lytechinus pictusor*	4.6
UPM-Kymmene Kraft	20000220	*Lytechinus pictusor*	2.96
UPM-Kymmene Kraft	19970916	*Menidia beryllina*	>72
UPM-Kymmene Kraft	19980407	*Menidia beryllina*	>73
UPM-Kymmene Kraft	19980810	*Menidia beryllina*	>67
UPM-Kymmene Kraft	19990321	*Menidia beryllina*	>67
UPM-Kymmene Kraft	19990906	*Menidia beryllina*	>67
UPM-Kymmene Kraft	20000220	*Menidia beryllina*	>70